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LISOZIMA COMO ALTERNATIVA PARA MELHORAR O DESEMPENHO E MICROBIOTA INTESTINAL EM SUÍNOS E A EXPLORAÇÃO DAS CARACTERÍSTICAS DA MICROBIOTA ENTRE ANIMAIS DE BAIXO E ALTO DESEMPENHO EM AVES E SUÍNOS.

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LISOZIMA COMO ALTERNATIVA PARA MELHORAR O DESEMPENHO E MICROBIOTA INTESTINAL EM SUÍNOS E A EXPLORAÇÃO DAS CARACTERÍSTICAS DA MICROBIOTA ENTRE ANIMAIS DE BAIXO E ALTO DESEMPENHO EM AVES E SUÍNOS [1]

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RESUMO

Este documento descreve uma revisão sistemática para aves e suínos e um estudo testando a lisozima em suínos em crescimento. A revisão sistemática objetivou identificar os estudos focados nas características da microbiota intestinal associadas a animais de alto e baixo desempenho e descrever criticamente os estudos disponíveis na área. A estratégia de busca foi realizada utilizando o sistema PRISMA e "PICo". Buscas independentes foram realizadas para suínos e aves. Os critérios de seleção foram artigos completos publicados em revistas científicas; todas as fases de crescimento; e uma comparação de animais de alto e baixo desempenho em relação aos seus fenótipos. O banco de dados final de suínos e aves foi composto por 19 e 17 artigos, respectivamente. Dois resultados principais foram encontrados em ambas as bases de dados. Verificou-se que diferentes critérios foram usados para classificar animais de alto e baixo desempenho de crescimento, porém a maioria dos estudos (47%) optaram pelo consumo alimentar residual em ambas as bases de dados. Houve alta variabilidade nas conclusões dos artigos que compuseram ambas as bases de dados, no entanto a maioria encontrou respostas semelhantes (84% e 82%, respectivamente) quanto à existência da relação entre microbiota intestinal e desempenho de crescimento do hospedeiro. No segundo estudo, 72 suínos machos castrados (40.6 ± 2.59 kg) foram avaliados durante 28 dias. Os suínos foram alimentados sob seis tratamentos: 0, 16, 32, 48, 64 e 80 mg/kg de lisozima na dieta, com o objetivo de testar os efeitos desta enzima no desempenho, composição corporal, balanço de nutrientes, perfil bioquímico sanguíneo e microbiota intestinal. Os animais diminuíram ($P < 0,05$) o consumo de ração,

[1] Tese de Doutorado em Zootecnia - Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (118p.), março de 2023.

enquanto melhoraram ($P < 0,05$) ganho médio diário, eficiência alimentar e deposição proteica à medida que a lisozima dietética aumentava. Além disso, a suplementação com lisozima melhorou a eficiência da utilização de lisina e treonina, e de N e P ($P \leq 0,01$). Porém, não houve alterações nas populações da microbiota intestinal e fecal com o aumento dos níveis de lisozima. O nível ótimo da lisozima para eficiência alimentar foi de 60 mg/kg, enquanto para ganho médio diário e melhor eficiência de utilização de N foi de 40 e 50 mg/kg de lisozima na dieta, respectivamente ($P \leq 0.01$). Portanto, para a revisão sistemática, a microbiota intestinal juntamente com fatores abordados, podem explicar em parte as diferenças no desempenho zootécnico de suínos e aves com alta e baixa eficiência alimentar, e por outro lado a lisozima melhorou o desempenho animal, composição corporal e o balanço de nutrientes.

Palavras-chave: Aditivo alimentar. Enzima. Microbiota. Muramidase. Revisão sistemática.

LYSOZYME AS AN ALTERNATIVE TO IMPROVE INTESTINAL PERFORMANCE AND MICROBIOTA IN SWINE AND THE EXPLORATION OF MICROBIOTA CHARACTERISTICS BETWEEN LOW AND HIGH GROWTH PERFORMANCE ANIMALS IN POULTRY AND SWINE[2]

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ABSTRACT

This study describes a systematic review for poultry and pigs and a study testing lysozyme in growing pigs. The systematic review aimed to identify studies focused on the characteristics of the intestinal microbiota associated with high and low growth performance animals and critically describe the studies available in the area. The search strategy was performed using the PRISMA and "PICo" systems. Independent searches were performed for swine and poultry. The selection criteria were complete papers published in scientific journals; all phases of growth; and a comparison of high and low growth performing animals. The final pig and poultry database consisted of 19 and 17 selected papers, respectively. We can highlight two major results for both databases. First, it was found that different criteria were used to classify animals with high and low growth performance, but most studies (47%) opted for residual feed intake in both databases. And second, the main conclusions of each study and for each database were presented. There was variability in the conclusions for both databases, most found similar conclusive answers (84% and 82%, respectively) regarding the existence of a relationship between microbiota and host growth performance. In the second study, 72 barrows (Yorkshire x Landrace) were used for 28 days. The pigs were fed under six different treatments: 16, 32, 48, 64, and 80 mg of lysozyme/kg diet, with the objective of testing this enzyme in performance, composition, nutrient balance, blood profile, intestinal microbiota, and optimal level of lysozyme. Animals decreased ADFI, while improved ADG, G:F, and PD as dietary lysozyme increased. In addition, it improved the efficiency of utilization of lysine and threonine, and of N and P ($P \leq 0.01$). There were no changes in animal microbiota populations with increasing levels of lysozyme. The optimal level

[2] Doctoral Thesis in Animal Science - Animal Production, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (118p.), March 2023.

of lysozyme for G:F was 60 mg/kg, while for ADG and N utilization efficiency, it was between 40 and 50 mg/kg of lysozyme in the diet ($P \leq 0.01$). This suggests that lysozyme may improve growth performance and nutrient balance, and if the objective is to maximize G:F, the optimal inclusion level of lysozyme is 60 mg/kg. However, if the objective is to minimize the inclusion of enzymes and improve the efficiency of ADG and N utilization, 40 to 50 mg/kg of feed is recommended.

Keywords: Enzyme. Feed additive. Microbiota. Muramidase. Systematic review.

LISTA DE ABREVIATURAS

AA	Amino Acids
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
DM	Dry Matter
FCR	Feed Conversion Ratio
FE	Feed Efficiency
LipD	Lipid Deposition
N	Nitrogen
P	Phosphorus
PD	Protein Deposition
RFI	Residual Feed Intake
SID	Standardized Ileal Digestible

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1 INTRODUÇÃO

A suplementação dietética com enzimas exógenas é uma prática comum na nutrição animal (JO *et al*., 2012; VELASQUEZ-DE LUCIO, 2021). As enzimas desempenham um papel importante na disponibilização de frações não digeríveis da dieta para absorção pelo animal e na redução dos fatores antinutricionais dos ingredientes. A suplementação de enzimas pode otimizar o valor nutricional das dietas, reduzindo assim o custo da alimentação, melhorando o desempenho zootécnico dos animais e reduzindo a poluição ambiental (ADEOLA; COWIESON, 2011; MEALE *et al*., 2014).

Além das enzimas exógenas comumente utilizadas na nutrição animal, há algumas enzimas com um papel considerável em aspectos clínicos, bem como na melhoria da saúde intestinal dos animais. Um dos compostos é a lisozima, tem como alvo componentes presentes no lúmen intestinal e pode ser considerada um aditivo alimentar (COOPER *et al*., 2014; LONG *et al*., 2016; SAIS *et al*., 2020). A lisozima é uma 1,4-β-N-acetilmuramidase que cliva enzimaticamente uma ligação glicosídica no componente peptidoglicano das paredes celulares bacterianas, o que resulta na perda da integridade da membrana celular e morte celular (ELLISON, 1991).

Vários estudos exploraram as vantagens do uso de lisozima isoladamente ou em associação com outros antibióticos, para uso em rações de aves (XIA *et al.*, 2019), suínos (LONG et al., 2015), bovinos (SMULSKI *et al*., 2020), peixes (KUMARESAN *et al*., 2015) e coelhos (EL-DEEP *et al*., 2021). A lisozima pode ser utilizada em animais de produção, como aves e suínos, com o objetivo principal de substituir melhoradores de crescimento em banimento (OLIVER; WALLS, 2015; ABDEL-LATIF, 2017). Em suínos, a lisozima tem potencial para melhorar desempenho e morfologia intestinal (MAY *et al*., 2012; OLIVER; WELLS, 2014; WELLS *et al*., 2015; LONG *et al.*, 2016; ZOU *et al*., 2019). Em aves, a melhora no desempenho também foi encontrada e reduções em bactérias patogênicas, como por exemplo, Clostridium perfringens (LIU *et al.*, 2010; FISININ, 2014). No entanto, mais estudos são necessários para melhor entender seus efeitos, em particular com as novas moléculas sintéticas de lisozima.

Uma breve revisão sistemática sobre lisozima foi realizada. Esta revisão sistemática mostrou que apenas um estudo com a fonte de lisozimas fúngicas transgênicas foi testado em

suínos. Portanto, nossos estudos serão os segundos a usar lisozima fúngica transgênica em dietas para suínos e os primeiros a estudar fatores que afetam a variabilidade no desempenho de crescimento dentro do mesmo tratamento (mesma dosagem) observado para aditivos alimentares. Neste sentido, é crucial que o primeiro passo para testar o potencial deste aditivo alimentar seja determinar a dose que maximiza o desempenho zootécnico em animais em crescimento, e entender sua ação a favor da saúde intestinal e principalmente dos fatores de variação.

2 REVISÃO BIBLIOGRÁFICA

2.1 ADITIVOS ALIMENTARES NA PRODUÇÃO ANIMAL

Os aditivos alimentares receberam muita atenção nos últimos tempos, especialmente na suinocultura. Entre os diversos mecanismos de ação associados aos aditivos, podem ser citados melhora na resposta imune dos suínos, redução da carga patogênica no intestino suíno, estímulo ao estabelecimento de microrganismos intestinais benéficos e estímulo a função digestiva (DE LANGE, 2010). As enzimas exógenas estão entre os aditivos alimentares mais utilizados. Estes têm se destacado nos últimos anos por melhorar o valor nutricional dos alimentos nas dietas de suínos (DE LANGE, 2010). Isso é alcançado por meio de vários mecanismos, incluindo a quebra de fatores antinutricionais presentes nos ingredientes da ração, eliminação do efeito de encapsulamento de nutrientes, quebra de ligações químicas específicas em matérias-primas que não seriam quebradas por enzimas endógenas, e complementação das enzimas produzidas por animais jovens (DANICKE, 1997).

Além das enzimas exógenas comumente utilizadas na nutrição animal, existem algumas enzimas com um papel considerável em aspectos clínicos, bem como na melhoria da saúde intestinal dos animais. Nesta área em particular, um dos compostos mais promissores é a lisozima, que é uma pequena enzima que ataca as paredes celulares protetoras das bactérias (KUROKI, 1993).

2.2 A ENZIMA LISOZIMA

A lisozima é uma 1,4-β-N-acetilmuramidase que cliva enzimaticamente uma ligação glicosídica no componente peptidoglicano das paredes celulares bacterianas, o que resulta na perda da integridade da membrana celular e morte celular (ELLISON, 1991). Além disso, os produtos de hidrólise são capazes de aumentar a secreção de imunoglobulina A (IgA), a ativação de macrófagos e a eliminação rápida de patógenos bacterianos (CLARKE, 2010).

A lisozima foi descoberta por Alexander Fleming com base na capacidade das secreções nasais de inibir o crescimento bacteriano (FLEMING, 1922). Trata-se, portanto, de uma enzima encontrada naturalmente em secreções corporais, como lágrimas, saliva e leite, além de ser encontrada em bactérias e fungos (JOLLES, 1984; TENOVUO *et al*., 1991).

A lisozima pertence a um grupo de substâncias que se difundem naturalmente, participando da estimulação imunológica do organismo. É assim que esta enzima é capaz de exercer uma atividade de estimulação para produzir anticorpos contra vários antígenos e melhorar a resistência contra infecções (SAHOO *et al*., 2012). Também é capaz de participar como parte dos mecanismos de defesa do organismo associando-se ao sistema de macrófagos e imunoglobulinas (GONG, 2014). Devido a essas características, a lisozima pode ser uma alternativa interessante e viável aos antimicrobianos em dietas de suínos.

2.3 MECANISMO DE AÇÃO DA LISOZIMA

A função antibacteriana da lisozima é produzida por ação bacteriolítica direta, ligando-se à parede celular bacteriana ou estimulando a função fagocítica dos macrófagos (SAHOO *et al*., 2012). Ela cliva a ligação glicosídica entre o ácido N-acetilmurâmico e a N-acetilglicosamina no peptidoglicano bacteriano, que é um componente importante das paredes celulares (BLAKE, 1965), portanto, fornece proteção contra infecções bacterianas.

A enzima utiliza um mecanismo de catálise covalente e catálise ácida, promovendo duas reações sucessivas de deslocamento nucleofílico. Liga-se a seis resíduos de Mur2Ac (ácido N-acetilmurâmico) e GlcNAc (N-acetilglucosamina) que se alternam em um típico peptidoglicano bacteriano, como o PDP ID 1 LZE. A ligação clivada é aquela entre o 4º e 5º resíduos que foram ligados pela enzima. O mecanismo mais aceito atualmente proposto (VOCADLO *et al.,* 2001) leva em consideração um mecanismo SN2. Nesse mecanismo, o 4º e 5º resíduos entram no sítio ativo da enzima e o aminoácido Asp52 da lisozima ataca o carbono anomérico de Mur2Ac. Este ataque libera o par de elétrons do carbono de GlcNAc, que se liga a um hidrogênio fornecido por Glu35. Neste ataque, a ligação é quebrada e o GlcNAc (juntamente com o restante do peptídeo ligado) é liberado da enzima, que ainda está covalentemente ligada a Mur2Ac. Essa ligação é quebrada pelo ataque da água, que cede um -OH para Mur2Ac e um H para Glu35, restaurando a enzima e liberando o restante do peptidoglicano (NELSON; COX, 2008).

No caso de bactérias gram-positivas, a lisozima causa lise alterando as propriedades das estruturas da superfície celular, destruindo a ligação glicosídica entre o ácido N-acetilmurâmico e a N-acetilglucosamina no peptidoglicano bacteriano, que é um importante componente da parede celular bacteriana. A composição química das paredes celulares das bactérias Gram-negativas difere daquela das bactérias gram-positivas. Este é composto por duas camadas, a camada interna

é composta por uma única camada de peptidoglicano e a externa por uma espessa camada de lipopolissacarídeo (SAHOO *et al*., 2012; GONG, 2014). A maioria das bactérias gram-negativas não é suscetível à ação da lisozima sozinha porque sua membrana externa impede que a enzima acesse a camada de peptidoglicano. No entanto, algumas lisozimas naturais que foram modificadas por várias técnicas, como a fermentação bacteriana, são ativas contra bactérias gram-negativas ao penetrar na camada externa (ELLISON III; GIEHI, 1991; SAHOO *et al*., 2012).

2.4 ABORDAGEM DE UMA REVISÃO SISTEMÁTICA SOBRE A ENZIMA LISOZIMA EM SUÍNOS

Uma revisão sistemática foi realizada sobre a suplementação de lisozima na produção de suínos, com foco em compreender melhor o que tem sido estudado sobre a suplementação da lisozima na produção de suínos e para explorar dados sobre as características da lisozima na saúde intestinal dos suínos. Esta revisão sistemática foi realizada seguindo um protocolo PRISMA detalhado (MOHER *et al*., 2009), com o objetivo de sintetizar informações relevantes sobre o escopo do estudo. Esta revisão sistemática foi realizada em agosto de 2020.

A base de dados inicial compreendia uma lista de 2.341 referências. Após a remoção de 530 referências duplicadas, 1.838 estudos permaneceram para análise. Desse conjunto de referências, 1.676 foram retiradas após a avaliação dos títulos, enquanto outras 20 foram retiradas após a leitura do resumo por não se enquadrarem em pelo menos um dos critérios de seleção. Esses critérios foram: (1) estudos originais de suplementação de lisozima; (2) estudos com suínos e (3) ano de publicação de 1990 a 2020.

Durante o processo de revisão, 47 artigos desenvolvidos com espécies diferentes de suínos foram excluídos. Outros 25 artigos foram excluídos por serem revisões de literatura e 33 artigos por avaliar a lisozima como parâmetro de resultado e não como suplemento dietético para suínos. Ao final dos procedimentos de avaliação, 26 estudos foram retidos para compor o banco de dados final.

Os artigos de pesquisa foram publicados de 2006 a 2020 em 21 periódicos diferentes, dos quais *Journal Animal Science* (4 artigos), *Transgenic Research* (2 artigos) e *Plos One* (2 artigos) tiveram a maior participação no banco de dados. Os estudos foram testados principalmente nos Estados Unidos (n=13), China (n=9) e Canadá (n=2).

A maioria dos estudos foi realizada na fase de desmame e pós-desmame. Em relação ao desafio sanitário, 65% dos estudos não impuseram um desafio sanitário, enquanto o restante (35%) desafiou os suínos com *Escherichia Coli* ou outra infecção induzida por colite. Considerando a forma de administração da lisozima, a mais utilizada foi a suplementação via dieta (65%). Para estudar o efeito da lisozima, os estudos usaram principalmente parâmetros sanguíneos (por exemplo, Imunoglobulinas IgA, IgG, IgM; Albumina e Globulina - 65% dos estudos), desempenho (58%), morfologia intestinal (54%) e expressão gênica (por exemplo, fator de necrose tumoral-α, IL-8 e IL- 10 - 35%). As principais fontes de lisozima suplementadas em dietas de suínos foram o leite de cabras transgênicas produtoras de lisozima humana (n = 9), seguido da clara de ovo (n = 8).

A fonte da qual a lisozima pode ser extraída em escala industrial é normalmente clara de ovo de galinha. Long *et al*. (2016) testaram a lisozima desta fonte e encontraram resultados positivos em relação ao desempenho, saúde intestinal e imunidade de leitões desmamados. Além disso, outros estudos usaram lisozima de leite transgênico ou de arroz transgênico e observaram efeitos benéficos da lisozima na morfologia intestinal (BRUNDIGE *et al*., 2008), microflora intestinal (MAGA *et al.*, 2006) e perfis de metabólitos (BRUNDIGE *et al.*, 2010). Ainda assim, tais fontes não são comercialmente viáveis devido ao custo, e novas lisozimas fúngicas transgênicas têm a capacidade de ser três vezes mais eficazes do que as fontes de clara de ovo (DR. J. ESCOBAR, comunicação pessoal).

Nossa revisão sistemática mostrou que apenas um estudo com a fonte de lisozimas fúngicas transgênicas foi testado em suínos. Portanto, nossos estudos serão os segundos a usar lisozima fúngica transgênica e os primeiros a estudar fatores que afetam a variabilidade no desempenho de crescimento dentro do mesmo tratamento (mesma dosagem) observado para aditivos alimentares. Neste sentido, é crucial que o primeiro passo para testar o potencial deste aditivo alimentar seja determinar a dose que maximiza o desempenho de crescimento em animais em crescimento, e entender sua ação a favor da saúde intestinal e principalmente dos fatores de variação.

2.5 A UTILIZAÇÃO DA LISOZIMA COMO ADITIVO ALIMENTAR

Vários estudos exploraram as vantagens do uso de lisozima isoladamente ou em associação com outras proteínas ou antibióticos, para uso em rações de aves, suínos, bovinos

(SMULSKI *et al*., 2020), peixes (KUMARESAN *et al*., 2015) e coelhos (EL-DEEP *et al.*, 2021). A lisozima pode ser utilizada em animais de produção, como aves e suínos, com o objetivo principal de substituir promotores de crescimento em banimento (OLIVER; WALLS, 2015; ABDEL-LATIF, 2017). Em suínos, May *et al*. (2012) publicaram um estudo em leitões de 10 dias usando uma dieta líquida suplementada com lisozima e demonstraram que há uma melhora no crescimento de suínos em resposta ao consumo da enzima. Houve também melhora da morfologia do intestino delgado e diminuição da prevalência de *Campylobacter* no trato gastrointestinal. Oliver e Wells (2014) também relataram a melhora na eficiência alimentar em suínos. Neste estudo, a lisozima melhorou a eficiência alimentar em cerca de 8% em comparação com suínos que não receberam dieta suplementada com lisozima, e semelhante aos suínos que consumiram dietas tratadas com antibióticos. O resultado de que a lisozima melhorou a taxa de crescimento e pode ser eficaz no controle de patógenos, quando alimentado em rações iniciais, foi confirmado por outros artigos nos quais a lisozima foi administrada na dieta em uma concentração de 100-120 mg/kg (OLIVER; WELLS, 2014; WELLS *et al*., 2015; LONG *et al*., 2016; ZOU *et al*., 2019). Estudos sobre as variações na microbiota intestinal também têm sido exploradas. Em porcas, a imunidade sérica e perfis de metabólitos do leite materno mediados pela suplementação de lisozima foi testado (ZHOU *et al*., 2018). Esses autores mostraram que a suplementação de lisozima pode efetivamente melhorar a composição, as funções metabólicas e os fenótipos da microbiota intestinal da porca e beneficiar as porcas com melhor estado imunológico e composição do leite materno.

Em aves, um experimento realizado por Liu *et al*. (2010) em frangos de corte constatou que ao adicionar lisozima à ração, houve melhora na taxa de conversão alimentar, redução significativa na concentração de *Clostridium perfringens* no íleo e nos escores de lesões intestinais, bem como inibição do supercrescimento de *Escherichia Coli* e *Lactobacillus* no íleo. Além disso, outro estudo em frangos de corte mostrou que o uso de lisozima reduz o consumo de ração, o percentual de gordura, melhora a digestibilidade e o ganho de peso diário (FISININ, 2014). Em outro artigo, frangos de corte foram alimentados com uma dieta inicial (1-21 dias) e uma dieta de crescimento (22-42 dias) suplementada com 0 (controle), 40, 100 ou 200 ppm de lisozima ou 400 ppm de flavomicina como controle antibiótico por 6 semanas. A administração de lisozima não contribuiu significativamente para o crescimento dos frangos de corte. Não foram encontradas diferenças significativas na diversidade e composição da flora bacteriana e

fúngica na microbiota cecal de frangos nos diferentes grupos de dieta. No entanto, a suplementação de lisozima levou a um enriquecimento significativo de genes envolvidos na síntese/degradação de membranas externas bacterianas e paredes celulares, transporte de substrato entre células e processos metabólicos de carboidratos, possivelmente promovendo o metabolismo de carbono e energia da microbiota cecal (YUN *et al.,* 2019).

No entanto, poucos estudos estão disponíveis na literatura sobre o uso da lisozima na variabilidade animal e mais estudos são necessários para melhor entender seus efeitos, em particular com as novas moléculas sintéticas de lisozima.

2.6 VARIABILIDADE ANIMAL

A variabilidade dentro dos tratamentos em um estudo pode ser grande. Essa variabilidade resulta das diferenças entre os animais quanto à genética, idade e peso (fatores intrínsecos). Além disso, fatores externos que influenciam o desempenho animal e as demandas de nutrientes (fatores extrínsecos) podem desempenhar um papel significativo. Cada animal responde de maneira diferente a esses efeitos, resultando em maior variabilidade entre os animais (WELLOCK *et al*., 2004). Considerar a variabilidade dentro e entre animais em programas nutricionais é crucial para avaliar a resposta biológica de suínos (KNAP, 2000; HAUSCHILD *et al*., 2010).

Além da variabilidade genética, a variabilidade no desempenho animal pode ser desencadeada por vários fatores, como a nutrição no início da vida (BIKKER *et al*., 1996) ou o estado sanitário dos animais que está associado a respostas fisiológicas ao estresse (DÉSIRÉ; BOISSY; VEISSIER, 2002). Além disso, o estado sanitário pode influenciar a utilização eficiente de nutrientes (RAKHSHANDEH *et al*., 2014) e, portanto, aumentar a variabilidade da ingestão de nutrientes. No caso de estudos de teste de aminoácidos, a disponibilidade de aminoácidos pode desafiar o metabolismo e resultar em aumento da variabilidade devido à mudança na eficiência energética e proteica em suínos alimentados com dietas limitantes em aminoácidos. Recentemente, discussões têm ocorrido para determinar se a microbiota pode desempenhar um papel importante no desempenho do crescimento animal, sendo parcialmente responsável pela variabilidade observada entre os suínos (QUAN *et al.*, 2020; JIANG *et al*., 2021). Um dos estudos apresentados nesta tese está focado em entender a variabilidade associada a todos os

fatores discutidos acima, para entender quais são os fatores que contribuem para um animal ser considerado um animal de “baixo” ou “alto” desempenho a um determinado tratamento.

2.7 INTERAÇÃO MICROBIOTA X HOSPEDEIRO

A microbiota pode desempenhar um papel significativo na saúde e no metabolismo do hospedeiro e o trato digestivo suíno fornece o habitat apropriado para muitas espécies microbianas (DUARTE *et al.*, 2022). A microbiota intestinal contribui para várias funções fisiológicas (SALZMAN *et al.*, 2007; LEE; HASE, 2014; MARCHESI *et al*., 2016), como funções protetoras (deslocamento de patógenos, competição por nutrientes, competição por receptores, produção de fatores antimicrobianos), funções estruturais (fortificação da barreira do trato gastrointestinal, indução de imunoglobulina IgA, *tight junctions*, desenvolvimento do sistema imunológico [PENG *et al*., 2021]) e funções metabólicas (fermentar resíduos dietéticos não digeríveis, sintetizar vitaminas, controlar a diferenciação e proliferação das células epiteliais intestinais, absorção de íons [GHOSH *et al*., 2021]).

Além disso, a microbiota intestinal contribui para a regulação da homeostase do hospedeiro, contribuindo para uma ótima digestão e absorção, regulação do metabolismo energético, prevenção de infecções da mucosa e modulação do sistema imunológico (WILLING; VAN KESSEL, 2010; UPADHAYA; KIM, 2022). Nesse caso, é importante que as interações hospedeiro-microbiota resultem em simbiose adequada para que ambas as partes recebam benefícios mútuos. Muitos fatores envolvidos, como mudanças nas práticas de alimentação, estresse (por exemplo, térmico, desmame, transporte, reagrupamento, superlotação [BURKHOLDER *et al*., 2008; NOWLAND *et al*., 2019; WEI *et al*., 2021]) e más condições de manejo e higiene, podem resultar em comprometimento da microbiota do trato gastrointestinal, o que pode afetar negativamente a funcionalidade do sistema de defesa local do hospedeiro. A microbiota suína pode ser afetada por muitos fatores, como estresse, dieta, práticas de manejo e compostos antimicrobianos que podem ser fatores-chave na patogênese de muitos distúrbios digestivos (KNECHT *et al.*, 2020). Portanto, uma microbiota normal, estável e diversificada, bem como uma barreira intestinal intacta e eficaz são necessárias para manter a funcionalidade gastrointestinal ideal.

As mudanças na população da microbiota intestinal por meio da lisozima podem ir além dos benefícios de manter um ecossistema equilibrado, mas também restaurar o sistema

imunológico do hospedeiro (DENG *et al*., 2021) responsável por melhorar a resposta imune e melhorar o desempenho do crescimento animal. Esta hipótese é parte do tema central explorado nesta tese.

2.8 ESTRUTURA DA TESE

Os estudos apresentados nesta tese fazem parte de um grande projeto de pesquisa sobre o uso do aditivo alimentar. A lisozima, por ser uma alternativa potencial à substituição ou redução dos antibióticos, atua de forma diferente dos mesmos frequentemente usados pela indústria para profilaxia. No entanto, o objetivo desses estudos iniciais não foi estudar a ação da lisozima em substituição aos antibióticos, mas sim entender sua ação a favor da saúde intestinal. Além disso, os estudos foram planejados de modo a considerar a interação do aditivo com a variabilidade naturalmente presente nas populações de suínos. Esta é uma característica extremamente relevante, mas que é muitas vezes ignorada em sistemas convencionais de produção ou pesquisa.

A partir disso, a tese está estruturada em um artigo de revisão sistemática que aborda a associação das características da microbiota intestinal considerando diferentes fenótipos entre baixo e alto desempenho. Apesar do foco principal da tese ser o estudo em suínos, optou-se por expandir essa abordagem também para os frangos de corte, visto que há uma complementariedade importante entre as áreas. Posteriormente, será apresentado o artigo que aborda o efeito da lisozima em suínos na fase de crescimento.

3 HIPÓTESES E OBJETIVOS

O objetivo central da pesquisa é avaliar o efeito potencial antibacteriano e anti-inflamatório de uma nova fonte de lisozima, para verificar sua capacidade de promover a saúde intestinal em suínos em crescimento, e o estudo das propriedades metabólicas, microbianas e nutricionais, fatores estes que estão potencialmente envolvidos na variação da resposta observada em suínos à ingestão nutricional e suplementação com lisozima. A hipótese principal é de que o uso da lisozima pode reduzir o risco de diarreia, melhorar o desempenho de crescimento, o balanço de nutrientes e a composição corporal, a microbiota e integridade intestinal de suínos em comparação com suínos não alimentados com dietas suplementadas.

Os objetivos específicos são:

- Explorar as características da microbiota intestinal entre animais com baixo e alto desempenho em aves e suínos a partir da revisão sistemática, como forma de entender melhor a variação existente entre grupos e identificar pontos críticos nesta área do conhecimento.

- Determinar o nível ideal de inclusão de lisozima na dieta para maximizar o desempenho do crescimento.

- Estudar o efeito da alimentação de suínos com dietas suplementadas com lisozima no desempenho de crescimento, composição corporal, composição da microbiota intestinal e integridade intestinal de suínos.

- Investigar como os fatores estudados contribuem para a variabilidade observada na composição corporal e desempenho animal.

CAPÍTULO II

Association of gut microbiome features with performance phenotypes in pig and poultry: a systematic review.

Este capítulo é apresentado de acordo com as normas de publicação da **Plos One.**

ASSOCIATION OF GUT MICROBIOME FEATURES WITH GROWTH PERFORMANCE PHENOTYPES IN PIG AND POULTRY: A SYSTEMATIC REVIEW

ABSTRACT

Gut microbiota is one of the key links between animal productivity and disease. Despite recent efforts, the extent to which the gut microbiome impacts the host's growth performance is not yet well understood in pig and poultry production. The objective of this study was to identify the studies focusing on the gut microbiome features associated with animals of high and low growth performance phenotypes, and to critically describe the studies available in the area. The search strategy was planned and carried out to identify as many studies as possible on the subject using the PRISMA statement. The first search was conducted in November 2021 and the entire selection procedure was applied to this dataset. Later, a new search was performed in June 2022 considering only the new publications in the area. Original peer-reviewed studies published in scientific journals available in PubMed, Scopus, and Web of Science were considered. Independent searches were performed for pig and poultry production systems. The strategy "PICo" was applied to build the research question. The selection criteria were stated as (i) full papers published in scientific journals; (ii) all growing phases; and (iii) a comparison of high and low growth performance animals. Pig and poultry final database were composed of 19 and 17 papers selected, respectively. Different criteria were used when classifying the animal phenotypes as high and low growth performance, but most studies (47%) also used the residual feed intake for both databases. In both database,

there were variability in the conclusions, most found similar conclusive answers (84% and 82%, respectively) regarding the existence of the relationship between microbiota and host growth performance. Furthermore, these studies have shown that there are differences in the composition of the microbiota between the two groups of animals (high and low growth performance) and that they could be potential use to distinguish individuals for growth efficiency. However, the specific markers differed greatly from among studies. Although there is variability in methodologies, selection criteria and conclusions for determining the microbiota and many factors affecting it, which may be a limiting factor and more studies are needed, this knowledge area already consolidated can potentially be directed in the future to manipulate the intestinal microbiome to improve feed efficiency in swine and poultry production. If successful, this has the potential to reduce production costs and the environmental impact of both animal productions.

Keywords: animal variability; high and low performing; microbiota

INTRODUCTION

The digestive system is certainly known for its classic role in digesting food and absorbing nutrients. However, recent research linked gut function to other numerous aspects of health, from immunity to chronic illnesses (Vighi et al., 2008). For that reason, the term 'gut health' has been increasingly used in many areas, including in animal sciences.

Gut health is a very complex term, which may include the structure and function of the gastrointestinal tract barrier, normal and stable microbiota, good immune status, in addition to effective digestion of food and absorption of nutrients (Celi et al., 2017). Understanding the complex mechanisms that maintain gut health is very difficult, but the characterization of the gut microbiota has been the subject of several studies, resulting in increased availability of several biomarkers that can potentially be used to assess intestinal health and functionality (Bischoff, 2011).

The great improvement in the understanding of the collection of microorganisms residing in the gastrointestinal tract also provided clear evidence that gut microbiota is one of the key links between animal productivity and disease (Celi et al., 2019). Despite recent efforts, the extent to which the gut microbiome impacts the host’s performance is not yet well understood in pig and poultry production (Conway, 1994). The objectives of this study were (i) to identify the studies focusing on the gut microbiome features associated with animals of high and low-performance phenotypes and, (ii) to critically describe the studies available in the area, as well as their main contributions to the research area using well established systematic-review practices.

MATERIAL AND METHODS

This systematic review was based on structured and elaborated research performed using online search methods. The search strategy was planned and carried out to identify as many studies as possible on the subject using the PRISMA statement. Papers were rigorously selected and those focusing on the gut microbiome features associated with animals of high- and low-performance phenotypes (linked with the growth traits, e.g., body weight, weight daily gain, or feed efficiency) on pig production and poultry were further evaluated.

Independent searches were performed for pig and poultry production systems. The strategy “PICo” was applied to build the research question by identifying “Population” (database 1: “pig”; database 2: “poultry”), “Interest” (“gut microbiome”), and “Context” (“performance responses”) for both searches. Alternative terms for population and interest were listed using synonymous words in English to compose the final search strategy. The context was applied later (through full-text reads) to avoid missing any study in which the response was not mentioned among the main terms (title, abstract, and keywords). The final search terms were:

Database 1:

(pig OR pigs OR swine OR sow OR gilt OR boar OR barrow OR piglet) AND (“gut microbiome” OR microbiota OR microbiome) AND (“growth performance” OR “feed efficiency” OR “performance responses” OR “residual feed intake” OR “low residual feed intake” OR “high residual feed intake” OR “gain : feed” OR “gain to feed” OR “feed conversion”)*

Database 2:

(poultry OR broiler OR broilers OR chicken OR chickens) AND ("gut microbiome" OR microbiota OR microbiome) AND ("growth performance" OR "feed efficiency" OR "performance responses" OR "residual feed intake" OR "low residual feed intake" OR "high residual feed intake" OR "gain : feed" OR "gain to feed" OR "feed conversion").

The first search was conducted in November 2021 and the entire selection procedure was applied to this dataset. Later, a new search was performed in June 2022 considering only the new publications in the area. Original peer-reviewed studies published in scientific journals available in PubMed, Scopus, and Web of Science were considered. A snowball approach using forward (e.g., databases) and backward research methods (e.g., direct journal search, reference lists, and studies listed in previously published reviews) was performed to increase the chance of including as many relevant studies as possible. No limitations on the geographic origin or year of publication were applied in both searches.

Each database was exported to the reference software (EndNote X9, Philadelphia, PA) used to organize references and manage part of the study selection. Duplicate references were identified and excluded. Studies were critically evaluated regarding their relevance and quality by examining titles and abstracts in relation to the objectives of the systematic review. The selection and evaluation of study eligibility for systematic review were performed by two reviewers individually. Studies that differed in terms of eligibility were reassessed by a third reviewer.

The selection criteria were stated as (i) full papers published in scientific journals; (ii) all growing phases; and (iii) a comparison of high- and low-performing animals. The quality of selected studies was further evaluated and information relevant to describe the proposed theoretical model was transferred to the pig and poultry spreadsheets. Finally,

cross-study comparisons were performed considering the subject, scope, and main results observed.

RESULTS

Pig database

The research process until obtaining the final pig database is described in **Figure 1A**. Articles obtained by online searches (2,358 references) were critically evaluated and successive exclusions were performed. The main exclusions (i.e., those more related to methodological aspects of the original studies) were performed when assessing the full text, when 59 references were eliminated (mainly by objectives, no direct comparison between high and low-performing groups). These studies did not present gut microbiome characteristics associated with high and low growth performance. The final list of 19 selected studies is described in **Table 1**.

Articles from 14 scientific journals composed the pig database, with most publications originating from Frontiers in Microbiology (4 papers) and Journal of Animal Science (3 papers). Most studies (53%) were conducted in China, with four studies developed in Ireland (16%), two in the Republic of Korea (11%), and one each in France (5%), Germany (5%), The Netherlands (5%) and Austria (5%). In addition, it is important to highlight that the studies differed from each other in terms of the genetics and sex of the pigs used in the trials. Still, 5 studies did not report the sex studied.

This subject is relatively new in the literature, with the first studies identified published in 2017 (4 studies, **Figure 2A**). Of the identified studies, 10 focused on the finishing phase, whereas 8 publications focused on the growing phase (**Figure 3A**). Although the post-weaning phases are recognized as the most critical for pig production,

only one study evaluated the relationship between intestinal microbiota and performance of recently weaned piglets.

The main methodologies used to assess the gut microbiome in pigs with high and low growth performance phenotypes are presented in **Table 2**. Different criteria were used when classifying the phenotypes as high and low growth performance, but most studies (47%) used the residual feed intake criteria.

Another important variation among the studies was the location of sample collection. Most studies (42%) collected only animal feces, while other studies collected digesta (26%), feces and digesta (21%), or mucosa and digesta (11%) to identify the microbiota and the inflammatory status of the animals. Studies that collected tissue samples also had variations in the segment collected. No study collected samples of the complete tract, but most studies used the ileum segment as a reference in the small intestine, while for the large intestine, the cecum was the reference segment.

Considering the method of analysis for these samples, 15 studies used 16s rRNA sequencing for taxonomic identification of bacteria, while 1 study used de novo metagenomics. Among them, only 3 studies compared the methodologies (16s rRNA x Shotgun metagenomic) for animals with different phenotypes of low and high-performance. In addition, for studies that were analyzed by 16s rRNA sequencing, the V3-V4 region of the bacterial 16S rRNA gene was the most studied (76%), followed by the V4-V5 regions (12%) and the V4 region (12%).

Besides the responses of the intestinal microbiota of the animals, which were evaluated in all articles reviewed (selection criteria), 15 studies also evaluated alpha diversity, and 18 studies evaluated beta diversity, being these parameters that contribute to understanding the number and abundance of species within a community

and the differences in species composition and their abundances between areas within a community, respectively (**Figure 4A**). Metabolic pathways (74%; e.g., “cysteine and methionine metabolism”, “carbohydrate metabolism”, “drug resistance: antimicrobial”) and growth performance responses (37%; e.g., average daily gain, feed intake, feed efficiency) were the parameters most studied, followed by volatile fatty acid analyses (32%; e.g., acetic, butyric, and propionic acids). However, other measures such as intestinal morphology (e.g., villus height, villus width, crypt depth), blood analysis (e.g., blood urea nitrogen, glucose, triglycerides, creatinine), immunological responses (e.g., cytokine, tight junction proteins, mucin), and carcass traits (e.g., muscle depth, fat, lean meat) remain poorly studied in the papers dealing with this topic, which can be highlighted as important measures to be addressed in future studies.

The main conclusions of each study are presented in **Table 3**. The studies had the same objective of further understanding the link between gut microbiota and animal growth performance. Although there is variability in the conclusions, most found similar conclusive answers (84%) regarding the existence of the relationship between microbiota and host performance. Furthermore, these studies have shown that there are differences in the composition of the microbiota between the two groups of animals (high and low growth performance) and that they could be potential use to distinguish individuals for growth efficiency. However, the specific markers differed greatly from among studies.

Poultry database

The research process until obtaining the final poultry database is described in **Figure 1B**. In the same way as the pig database, articles obtained by online searches

(1,507 references) were critically evaluated and successive exclusions were performed. The main exclusions (more related to methodological aspects of the original studies) were performed when assessing the full text when 5 references were eliminated (studies that did not present the characteristics of the gut microbiome associated with animals with high and low growth performance). The final list of 17 selected studies is described in **Table 4**.

In the total poultry database, 12 journals reported publications, with 4 papers being published in the Frontiers in Microbiology, 2 papers in the American Society for microbiology and 2 papers in Poultry Science. Most studies (29%) were conducted in Austria, with four studies developed in China (24%), three in Australia (18%), two in India (12%), and one each in the Republic of Korea (6%), United Kingdom (6%), and USA (6%). In addition, it is important to highlight that the studies differed from each other in terms of genetics and sex of the poultry used in the trials. As in the pig database, only one study did not report the sex of the animals.

The first studies identified published were in 2017 (4 studies, **Figure 2B**). Of the identified studies, 13 focused on the growing phase, while 4 publications focused on the finishing phase (**Figure 3B**). Although the initial phases are also recognized as the most critical for poultry production, no studies were observed that evaluated the characteristics of the intestinal microbiome associated with animals with high and low growth performance phenotypes in the early stages of bird rearing.

The main methodologies used to assess the gut microbiome in poultry with high and low-growth performance phenotypes are presented in **Table 5**. Different criteria were used when classifying the animal phenotypes as high and low growth

performance, but most studies (47%) also used the residual feed intake as in the pig database, followed by the variable feed conversion ratio (35%) as a selection criterion.

Another important variation among the studies was also the location of sample collection. Most studies (47%) only collected digesta, while other studies collected feces (23%), mucosa and digesta (18%), or feces and digesta (12%) to identify the microbiota of animals. Studies that collected tissue samples also had variations in the segment collected in poultry. No study collected samples of the complete tract, but most studies also used the ileum segment as a reference in the small intestine, while for the large intestine, the cecum was the reference segment as in the pig database, and only one study collected and analyzed the cloaca.

Considering the method of analysis for these samples for poultry, 15 studies used 16s rRNA sequencing for taxonomic identification of bacteria, while one study used shotgun metagenomic sequencing, and another study used *de novo* metagenomics. In addition, for studies that were analyzed by 16s rRNA sequencing, the V3-V5 region of the bacterial 16S rRNA gene was the most studied (29%), followed by the V1-V3 regions (18%), the V3-V4 region (18%) and the rest were regions V1-V5, V4-V5 and V4.

Besides the responses of the intestinal microbiota of the animals, which were evaluated in all articles reviewed (selection criteria), 13 studies also evaluated alpha diversity and beta diversity (**Figure 4B**). Relative abundance (100%, e.g., genus, phylum, family, and species level), growth performance responses (59%; e.g., average daily gain, feed intake, feed efficiency), and metabolic pathways (35%; e.g., carbohydrate, energy, and lipid metabolism) were the parameters in most studies. In addition, two studies evaluated the visceral organs differently from the pig database. However, other measures such as intestinal morphology (e.g., villus height, villus width,

crypt depth), volatile fatty acids (e.g., acetic, butyric, and propionic acids), immunological responses (e.g., cytokines, mucin, glucose transporter 2) remain poorly studied in the papers dealing with this topic, which can be highlighted as important measures to be addressed in future studies.

The main conclusions of each study are presented in **Table 6**. Most studies found similar conclusive answers (82%) regarding the existence of a relationship between microbiota and host. Furthermore, the same studies have also shown that there are differences in microbiota composition between the two groups of animals (high and low growth performance) and that they could potentially be adopted as biomarkers to improve growth performance or used to modify dietary strategies to improve growth performance of commercial birds. Again, comparable to the pig database, these potential biomarkers differed greatly among studies.

DISCUSSION

The availability of peer-reviewed publications focusing on the gut microbiome features associated with animals of high and low growth performance phenotypes of pig and poultry production systems has been explored more heavily in recent years. The first published studies were for the poultry production chain (2012) and later for pigs (2017). However, the availability of studies focusing on pig production has evolved a lot in the following years, especially after the first publication in 2017. In most research areas, the number of studies on poultry production is greater than the number of publications available on a comparable topic in pigs. However, the opposite was found in this systematic review, in which the number of studies per year was higher than in poultry studies, probably due to the physiological particularities of pigs, the greater complexity of the microbiota and intestinal health relationship, and for being a great model of study for human health.

The interest in investigating and understanding the characteristics of the gut microbiome associated with animals with high and low growth performance phenotypes stems from the variability in the growth performance responses of the animals, especially feed efficiency (FE), which is closely related to the gut microbiota (Singh et al., 2014; Yan et al., 2017). Concerns about feed costs in poultry and swine production have increased greatly in recent years. In response, improvements in FE of animals associated with a better understanding and manipulation of the intestinal microbiota can reduce production costs, in addition to reducing the environmental impact of these productions.

A variety of factors has been known to influence the gut microbiome of animals, including genotype (Turnbaugh et al., 2006), age (Niu et al., 2015), different regions of

the intestine (Kogut, 2022), and sex of the animals (Lee et al., 2017). It is important to highlight that several studies in both databases have mentioned some important factors (e.g., genetics and sex) in their methodologies as important characteristics to be considered in determining the contribution of the intestinal microbiota, as well as later in the animal growth performance in pigs and poultry production. Accordingly, host genetics has been suggested as an important factor in the determination of gut microbial composition (Turpin et al., 2016; Wen et al., 2021). Limited studies measured the contribution of inter-individual variability modulating the bacterial communities and the effect of the host on the establishment of the microbiota since conditions are difficult to standardize between individuals. However, it is largely unknown whether host genetics affect feed utilization through their ability to promote a stable microbial community in the gut or whether the two interact to influence feed efficiency (Wen et al., 2021). In this context, specific studies (e.g., genome-wide association) that have been widely used to analyze a multitude of complex traits, are now being used to study the link between the host and its microbial composition (Crespo-Piazuelo et al., 2019). In addition, sex or gender is also an important factor that gender-specific developmental properties are associated with different sex hormones that generally influenced metabolic processes, such as estrogen and androgen, which regulate the microbial composition and the activity of gut microbiota (Kanehisa et al., 2006; Varlamov et al., 2014).

Another important factor is the age or phase of the animals. Most studies evaluated the differences in phenotypes in relation to the intestinal microbiota in the final stages of production. The studies in the poultry area evaluated mainly in the growth phases, while the studies in the swine area were mostly developed in the finishing phase, one being in the post-weaning phase. Therefore, it is observed that there is a

need for further studies in younger animals for both databases, since the establishment of robust microbiota in the early life of animals is extremely important for growth as it is related to the development of intestinal functions and immune system (Kabat et al., 2014). This relationship between the immune and intestinal systems is closely related. For example, one of the strategies that the host utilizes to avoid an inflammatory response against the microbiota is to use the intestinal barrier, including the mucous layer and immunoglobulin (IgA), an antibody specialized in mucosal protection (Gutzeit et al., 2014) and produced locally by plasma cells present in the mucosal wall. Production of IgA is controlled by specifics cytokines by T-cells within the GALT (gut-associated lymphoid tissue) and by cytokine released from the mucosa. Within the GALT, the Th1 cytokines, interferon γ (IFN-γ), and tumor necrosis factor-β (TNF-β), downregulate IgA production, whereas the Th2 cytokines, interleukin (IL)-4, IL-5, IL-6, and IL-10, upregulate IgA production (Stokes, 2017). A balance between Th1 and Th2 response is necessary for maintaining normal IgA immune responses. IgA secreted into the gut lumen binds to the layer of mucus coating the epithelial surface, and it prevents the adherence of pathogens microorganisms (Gonnella et al., 1998).

Furthermore, pig gut microbiota shows dynamic composition and diversity which shifts over time and along the completely gastrointestinal tract (Isaacson R and Kim H.B, 2012). Weaning piglets are usually vulnerable to nutritional, and psychological stressors, leading to alterations of intestinal morphology, physiological function, and a shift in the intestinal microbiome (e.g., increased potential pathogens and diarrhea) and an excessive immune stimulation during this period has the potential to interrupt development and the long-term function of the gut's immune system and consequently growth performance (Yang, et al., 2016). Also, the microbial community in young chicks

changes with age, increasing its complexity, as mature birds develop more stable bacterial communities (Lu et al., 2003). Therefore, the relationship between community diversity, animal age, and FE over time is worthy of attention.

Most studies that compared microbiome data of the animals between high and low growth performance groups are based on a phenotypic selection of extreme animals in a population. Therefore, the growth performance of the animals may be evaluated by using the residual feed intake (RFI), feed conversion ratio, and others measures that are metrics of FE (Willems et al., 2013). However, several factors may contribute to differences in FE among animals. The intestinal microbiota is considered an important factor and could influence FE in several ways, such as variation in several physiological factors along the gastrointestinal tract, including nutrient digestibility and immune function (Vigors et al., 2016; Vigors et al., 2019).

The most used selection criterion among the studies was RFI for both databases. This selection criterion has been discussed in the literature for its advantages in relation to other criteria already consolidated in poultry and swine production (for example, by the feed efficiency itself, feed conversion, or body weight), precisely because it considers the biological mechanisms of the animals, defined as the difference between actual feed intake and predicted needs based on animal maintenance and growth (Koch et al., 1963). However, selecting animals for low residual feed intake (RFI) as a measure of feed efficiency has limited impacts on other production traits. In the review by Gilbert et al., 2017, which summarizes the results of INRA (Institut National de la Recherche Agronomique) the variations that should be considered for a more efficient selection between animals of low or high performance were well documented. Nitrogen utilization (as % of absorbed N) and protein deposition, two variables of great interest that express

production traits, resulted in similar rates between high and low growth performance animals (Barea et al. al., 2010; Labussière et al., 2015). Furthermore, the use of non-standard diets (eg, high or low fiber diets) which directly influence digestive efficiency does not seem to explain the variation between high and low growth performance animals. In this sense, the microbiota is often considered the main factor in variations between animals and is more associated with the different use of feed than as a direct effect. Therefore, these variables that are considered of extreme importance in animal production and that still seem uncertain as to their use for differentiation in animal drawing, it becomes challenging to use the RFI variable as an animal selection criterion. Considering variables such as direct measures (e.g., protein deposition or lipid deposition) to measure the variation between high and low growth performance should be considered as a great opportunity for future studies and not just the feed intake of the animals.

The differences between the sample collection sites for the identification of animal microbiota are another important point to be highlighted. In the swine database, most studies evaluated animal feces followed by samples in digesta. On the other hand, in the bird database, most studies evaluated digesta followed by fecal samples. This difference is probably related to the lower complexity of sample evaluation of collection of feces in pigs compared to the birds. Birds have the excretion system of urine and feces together, which makes it difficult to determine the microbiota. In addition, fecal sampling is a convenient and non-invasive sampling method that provides a reasonably good representation of the gut microbial communities (Ingala et al., 2018). The studies that collected tissue samples, had variations in the segment collected for both databases. In

both databases, the ileum was the tissue more collected in the small intestine, while the cecum was in the large intestine.

The composition of bacterial communities found in the different regions for both species' intestines might be judged as distinct ecosystems (Zhao et al., 2015; Shang et al., 2018). This difference may be mainly caused by the functional activities of the sections of the intestine, which are quite different. The small intestine is primarily responsible for the digestion and absorption of food, while the large intestine, especially the cecum, which has a high number of microorganisms is related to microbial fermentation (Zhao et al., 2015). Thus, different niche environments contribute to the difference of microbial composition in the lumen and mucosa. There are specific intestinal bacteria in the small intestine that actively participate in the digestion and metabolism of carbohydrates (El Kaoutari., 2013) and amino acids (Dai et al., 2010). The latter being very well documented by the review by Dai et al. (2010), in which they suggest that intestinal bacteria can significantly affect AA (amino acids) metabolism in pigs. Luminal bacteria in the small intestine can quickly utilize dietary AA and thus decrease AA supply to the epithelial cells, which might be a nutritional waste to the host. On other hand, the tightly attached bacteria could utilize the recycled ammonia for AA synthesis (Fuller and Reeds, 1998), which might provide extra AA to the host to meet the requirement. In broilers, there is evidence for the role of amino acids such as glutamine, arginine, and threonine have a positive effect on control permeability, promoting cell proliferation, and stimulating several metabolic pathways (Bortoluzzi et al., 2021). Furthermore, some dietary proteins in the small intestine may escape full enzymatic digestion and flow directly to the large intestine where microorganisms can ferment (Yang et al., 2019). As an example, Clostridiales were closely related to the

production of short chain fatty acids (SCFAs) and had certain anti-inflammatory effects in pigs (Martin-Gallausiaux et al., 2020). Jiang et al. (2021) study mentioned in this systematic review, also identified the enrichment of Clostridiales, including *Ruminococcus flavefaciens*, *Lachnospiraceae* spp., *Butyrivibrio proteoclasticus*, *Roseburia* spp., *Coprococcus eutactus* e *Eubacterium eligens* in pigs with high FE. In broiler study, Metzler-Zebeli et al. (2019b) observed that unclassified *Lachnospiraceae* genus *Ruminococcus* may have contributed to the higher cecal propionate concentration. The SCFAs are important fuels for intestinal epithelial cells (IEC) and regulate IEC functions through different mechanisms to modulate their proliferation, differentiation, as well as functions of subpopulations such as enteroendocrine cells, to impact gut motility and to strengthen the gut barrier functions as well as host metabolism (Martin-Gallausiaux et al., 2020). Thus, these suggest that possible differences in bacterial composition between segments would influence nutrient uptake and growth efficiency of the animals.

The studies also highlighted the differences in the analysis methods for determining intestinal microbiota. In both databases, the microbiota information is most often derived from partial sequencing of the bacterial 16S ribosomal RNA (rRNA) gene, a housekeeping gene in all bacteria (Woese, 1987). Sequencing the 16S rRNA gene has become a standard approach in bacterial taxonomic classification, due to its ease to generate phylogenetic information at high throughput (Wang et al., 2015). Furthermore, hypervariable regions of the 16S rRNA gene (V1 to V9) varied between studies in both databases and they are useful to study the variability of the microbiota. These variations in the choices of the genes studied can generate an erroneous understanding of the readings. And often, some regions may not be as effective at detecting variability. This is

because in the literature, the V3-V4 region proved to be useful for studying the variability of the microbiota. The ends of each read overlap and can be stitched together, and in a single run, it generates extremely high-quality, full reads of the full V3 and V4 region (Verschuren et al., 2018). Quan et al. (2019), Yang et al. (2017), and Jiang et al. (2021) were the only studies that briefly compared the shotgun metagenomic sequencing and 16S rRNA methodologies for investigate microbial composition. Although Shotgun metagenomic sequencing, unlike 16S rRNA sequencing, can read all genomic DNA in a specimen rather than just one particular area (Durazzi et al., 2021), the studies observed that the phylogenetic composition of the fecal microbiota determined by shotgun metagenomic sequencing was similar to the result obtained in the 16S rRNA gene sequencing. Firmicutes, Bacteroidetes, Spirochaetes, and Proteobacteria were the dominant phyla (Quan et al., 2019), *Prevotella, Lactobacillus,* and *Treponema* were the three most abundant genera (Yang et al., 2017), and Firmicutes, Bacteroidetes, Spirochaetes, and Proteobacteria were the most abundant phyla (Jiang et al., 2021). However, these samples were evaluated only in feces samples. There is no evidence of a direct comparison of these two analysis methodologies for samples in gut tissues between low and high-performance animals in both databases. In addition, variability in results regarding taxonomic levels was observed, inferring the difficulty of decision-making on which levels (e.g., genus, species, or family) to use for a given analysis methodology. In this sense, to gain new insight into the complex traits and underlying functional mechanisms in feed efficiency there is a need for further investigation in this area of knowledge.

The parameters evaluated in the microbiota studies with high and low growth performance in swine and poultry production were similar. However, analysis of alpha-

diversity, beta-diversity, and relative abundance were the parameters of greatest interest for both databases. Alpha diversity represents species within-habitat diversity, and beta diversity represents species between-habitat diversity. Both are helpful to evaluate the overall diversity of species comprehensively (Whittaker, 1972), while relative abundance explores the taxonomic distribution of the numerically abundant bacteria in each gut location or feces. There is a variety in the responses found for these parameters between studies for the same. For example, in chicken studies, these differences found are generally subtle between high and low RFI broilers, or even no difference found, and it is perhaps not surprising that the results are inconsistent across different studies. These apparently inconsistent results could be due to dietary and environmental differences or simply to the relatively low level of selection for birds between high and low RFI (e.g., number of animals between groups; Huang et al., 2021, and Lee et al., 2017). Likewise, studies in pigs have also shown divergent results. While some authors found no difference in alpha and beta diversity (McCormack et al., 2017; Metzler-Zebeli et al., 2018, Si et al., 2020), others found that pigs with lower FE had greater alpha diversity in their gut microbiota than pigs with higher FE (Wang et al., 2021) or greater alpha diversity higher for the heavier group than those for the lighter group (Han et al., 2017). However, some differences in composition associated with RFI or FE were found. Therefore, these divergences in the results in both databases clearly show us the great challenge we have in working with different groups in terms of performance. Even if there are non-controllable factors (e.g., intrinsic factors), there will be a need for future studies focused on standardizing responses to better understand what is desirable or not for different groups.

Finally, despite the different conclusions presented in each study in both species, the studies strongly evidenced that there is an interaction between microbiota and host and this interaction is dependent on intrinsic (e.g., genetics, age, and weight) and extrinsic (e.g., environment, nutrition) factors of the animals. As mentioned earlier, there is variability regarding the use of the criteria for selecting animals between studies (FE, RFI, daily weight gain, residual feed conversion), but regardless of the choice, all criteria were influenced by this interaction differently and significantly between groups (high and low-performance). It is important to remember that all the criteria used in the studies take into account mainly the feed intake of the animals. As previously mentioned, the importance of considering other parameters in the calculation (e.g., protein deposition and lipid deposition) would make the selection more accurate and judicious in detecting differences between animal growth performance. In addition, standardizing for a parameter would help to better understand the results regarding these differences and would facilitate decision-making for the production system.

Even though some studies have shown some possible links between intestinal microbiota and feed efficiency, there is still a need for greater understanding of the variability of the responses found regarding the influence on the composition of the animal microbiota. In this current review, we could see that most studies brought microbiota related responses in 84% and 82% for swine and poultry, respectively. Some bacteria are exclusively related to high-growth performance while others are related to low-growth performance. For example, bacteria from the phylum *Firmicutes* were related to high growth performance in both species (Han, et al., 2017; Du et al., 2020) and from the phylum Bacteroidetes to low growth performance in pigs (Stanley, et al., 2013). In poultry studies, there is greater variability in the responses found, but in general, the

phylum *Proteobacteria* were negatively correlated (Du et al., 2020) with growth performance. In fact, this variability in the answers makes it difficult to have a clear understanding for decision-making. There are bacteria from the same family that are positively correlated with growth performance while others have the opposite impact, and there is a need to study the differentiation of bacteria into species or subspecies. In this sense, further studies would be needed to confirm the causality of gut microbes with FE and to elucidate the possible mechanism of gut microbiome affecting pigs and poultry FE. As for the evaluation of the characteristics of the microbiota over time, it has been little explored by studies. In the swine database, only one study evaluated the composition of the microbiota between low and high-growth performance animals over time (McCormack et al., 2017), while two studies in the poultry database (Siegerstetter et al., 2018a; Siegerstetter et al., 2018b). Both databases evaluated fecal samples, which would facilitate the exploration of microbiota characteristics over time in high and low-growth performance animals for future studies. Overall, all studies found subtle differences in both the diversity and composition of the gut microbiota over time between low and high-growth performance animals. More research is needed to confirm the insights provided in these studies to improve understanding of the key changes that occur in the microbiota over time among different growth performance groups.

Overall, this study showed that there is a need for uniformity in the responses used to determine differences between high and low performing groups. Standardization in the experimental (e.g., sample collection site) and analytical (e.g., sequencing techniques) methods is also necessary. Studies focusing on young animals, should be considered when conducting new projects in this research area, as this is an important lack in the current literature. The diversity in the final answers between studies for both

databases deserve attention and future studies should be carried out for a possible complementarity of the answers found.

However, it is important to highlight these findings already bring us relevant insights in this area of knowledge, in which some groups of bacteria already have their potential for use as biomarkers in the future. In addition, these studies direct us to some viable alternatives for improvements in animal FE, modification of diet composition (for the use of specific nutritional additives that modulate the intestinal microbiota), and improvements in the efficiency of the use of animal metabolic pathways (animals classified as high growth performance have a greater ability to utilize dietary nutrients, energy saving mechanisms and moderation in the immune response).

CONCLUSION

This systematic review is one of the first to show a general approach of studies focusing on gut microbiome characteristics associated with high and low performing animals' phenotypes. The intestinal microbiota of the animals, together with the factors addressed, may partially explain the differences in the growth performance of pigs and birds with high and low feed efficiency. One of the main points is that there is significant variation between studies in relation to the selection criteria for determining animals with different phenotypes (high and low-performance). However, this study provided a potential set of methodological information in this area of knowledge for pig and poultry production. Although there is variability in methodologies for determining the microbiota and many factors affecting it and which may be a limiting factor and more studies are needed, this knowledge (techniques, approaches, and definitions) already consolidated can potentially be directed in the future to manipulate the intestinal microbiome to

improve feed efficiency in swine and poultry production. If successful, this has the potential to reduce production costs and the environmental impact of both animal productions.

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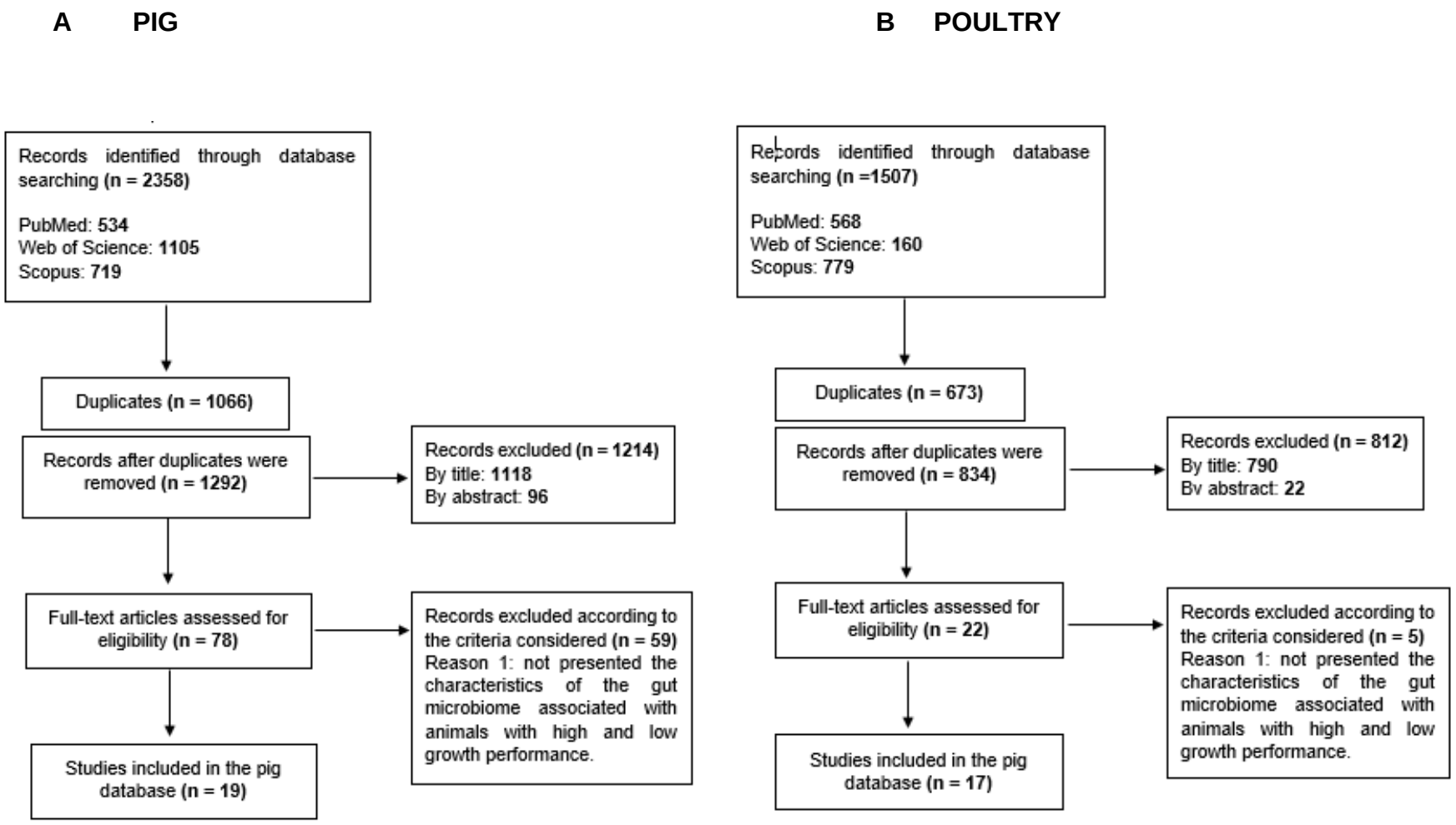

FIGURE 1. Study selection diagram for pig **(A)** or poultry **(B)** databases.

Table 1. Summary of the studies assessing the microbiota in pigs with high and low-performance in terms of country, genetics, and sex.

Code	Studies	Country	Genetic	Sex
1	Aliakbari et al., 2021	France	French Large White	Mixed
2	Han et al., 2017	Korea	Duroc x Large White x Landrace	-
3	Jiang et al., 2021	China	Duroc	Mixed
4	McCormack et al., 2017	Ireland	Large White x Landrace x (Maxgro)	Mixed
5	McCormack et al., 2018	Ireland	Large White x Landrace x (Maxgro)	Mixed
6	Oh et al., 2020	Korea	Landrace x Yorkshire x Duroc	-
7	Quan et al., 2018	China	Duroc × (Landrace × Yorkshire)	Female
8	Quan et al., 2019	China	Duroc x (Landrace x Yorkshire)	Female
9	Quan et al., 2020	China	Duroc x (Landrace x Yorkshire)	Female
10	Reyer et al., 2020	Germany	Large White x Landrace	-
11	Si et al., 2020	China	Duroc	-
12	Tan et al., 2017	China	Landrace	Female
19	Tan et al., 2018	China	Landrace	Female
13	Verschuren et al., 2018	The Netherlands	(Synthetic boar × [Large White x Landrace])	Mixed
14	Vigors et al., 2020	Ireland	Large White x Landrace x (Maxgro and line 37)	Male
15	Wang et al., 2021	China	Landrace	Female
16	Yang et al., 2017	China	Duroc	Mixed
17	He et al., 2018	China	Duroc x Large White x Landrace	Male
18	Metzler-Zebeli et al., 2018	Austria	Landrace	-

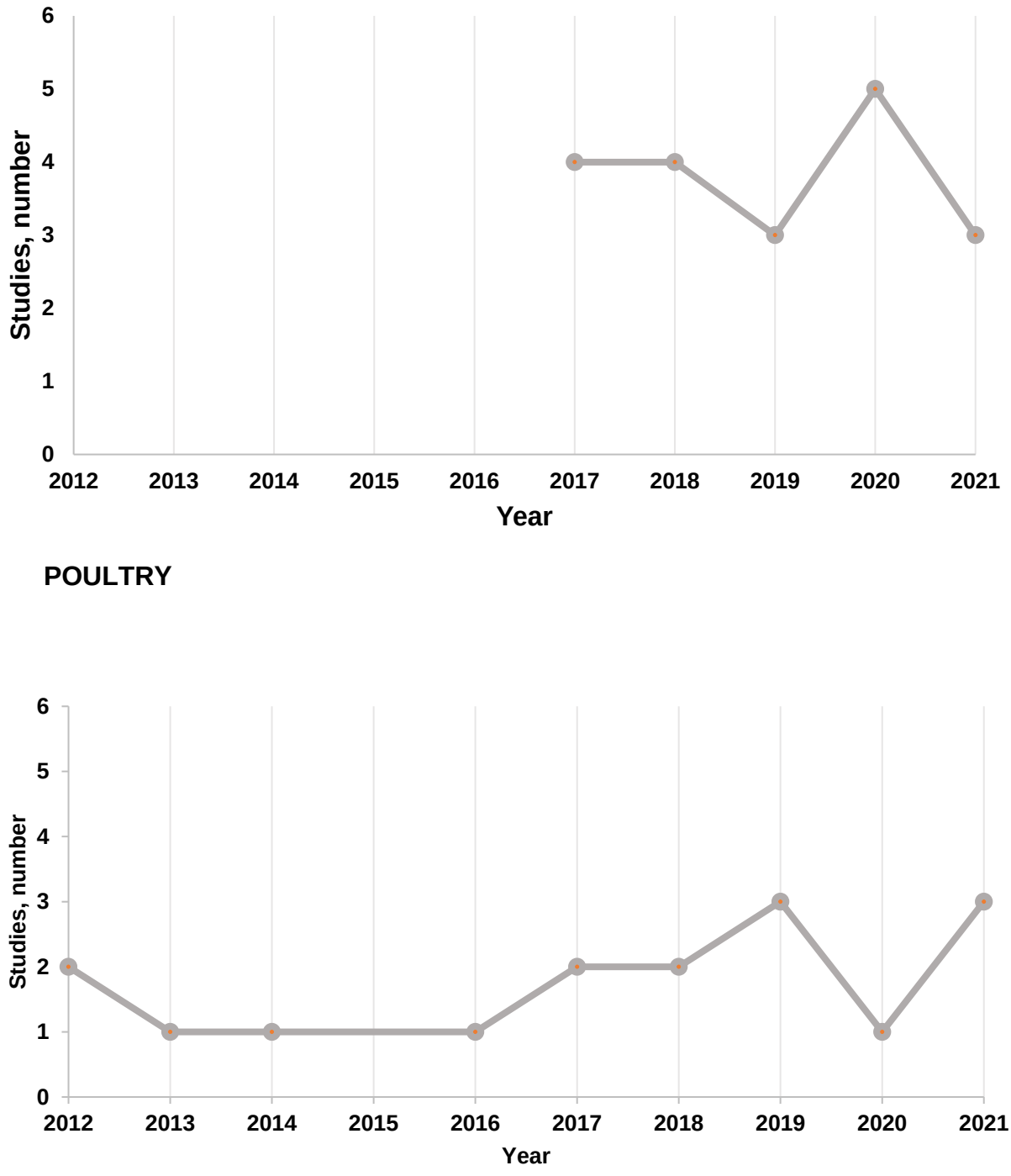

Figure 2. Year of publication of studies to assess the microbiota with high and low performance in pigs **(A)** or poultry **(B)**.

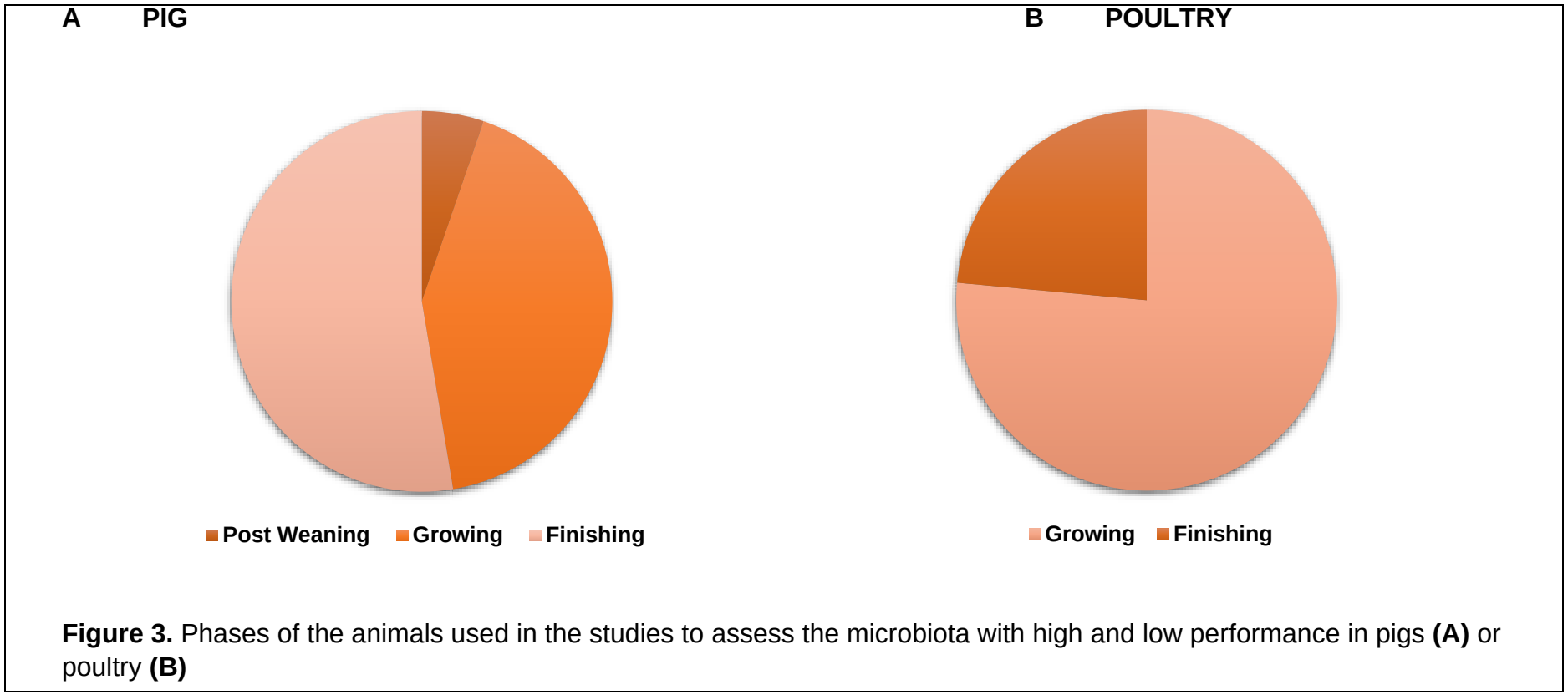

Figure 3. Phases of the animals used in the studies to assess the microbiota with high and low performance in pigs **(A)** or poultry **(B)**

Table 2. Summary of the methodologies applied to assess the microbiota in pigs with high and low-performance.

Code	Studies	Selection criteria[1]	Collection local	Intestinal tissue	Analysis method[2]	Region[3]
1	Aliakbari et al., 2021	RFI	Feces	-	16S rRNA	V3-V4
2	Han et al., 2017	BW	Feces	-	16S rRNA	V4
3	He et al., 2018	FE	Digesta	Cecum	16S rRNA	V3-V4
		ADG	Digesta	Cecum	16S rRNA	V3-V4
4	Jiang et al., 2021	RFI	Feces	-	16S rRNA; Shotgun	-
5	McCormack et al., 2017	RFI	Feces and Digesta	Ileum; Cecum	16S rRNA	V3-V4
6	McCormack et al., 2019	RFI	Feces and Digesta	Ileum; Cecum	16S rRNA	V3-V4
7	Metzler-Zebeli et al., 2018	RFI	Mucosa and Digesta	Caecum	16S rRNA	V3-V4
8	Oh et al., 2020	BW	Feces	-	16S rRNA	V3-V4
9	Quan et al., 2018	FCR	Luminal content	Ileum; Cecum; Colon	16S rRNA	V4-V5
10	Quan et al., 2019	FCR	Feces	-	16S rRNA	V4-V5
11	Quan et al., 2020	FE	Digesta	Ileum; Cecum; Colon	Shotgun	-
12	Reyer et al., 2020	RFI	Mucosa and Digesta	Ileum; Cecum	16S rRNA	V3-V4
13	Si et al., 2020	RFI	Feces	-	16S rRNA	V3-V4
14	Tan et al., 2017	FE	Digesta	Cecum	*de novo* metagenomics	-
15	Tan et al., 2018	FE	Feces and Digesta	Small intestine[4]; Colon	16S rRNA	V3-V4
16	Verschuren et al., 2018	FE	Feces	-	16S rRNA	V3-V4
17	Vigors et al., 2020	RFI	Digesta	Colon	16S rRNA	V3-V4
18	Wang et al., 2021	FCR	Feces and Digesta	Colon	16S rRNA; Shotgun	V3-V4
19	Yang et al., 2017	RFI	Feces	-	16S rRNA; Shotgun	V4

[1]Criterium selected to identify the different phenotypes in high and low-performance: RFI: residual feed intake; FCR: feed conversion ratio; BW: body weight; FE: feed efficiency; ADG: Average daily gain.
[2]Analythical method for bacterial taxonomic classification; 16S rRNA: 16S rRNA gene sequencing; Shotgun: Shotgun metagenomic sequencing.
[3]Hypervariable region of the bacterial 16S rRNA gene.
[4]Collection in all segments of the small intestine.

Table 3. Summary of the main subject and conclusions of the papers about microbiota versus high and low growth performance in pigs

Code	Study	Main study subject	Conclusion[1]
1	Aliakbari et al., 2021	Genetic, feed efficiency and gut microbiome	Part of the variability of the gut microbial community is under genetic control and has genetic relationships with FE, including diversity indicators.
2	Han et al., 2017	Body weight and intestinal microbiota	The level of microbial richness was higher in the microbiota of the heavier group than that in that of the lighter group, and several different bacterial phyla and genera that were differentially represented in the two groups were identified. The levels of genes related to several metabolic pathways were significantly different in the microbiota of the two groups.
3	He et al., 2018	Nutrient utilization and feed conversion ration	The presence of SCFA-producing bacteria in the caecum and increased muscular growth may contribute to the high FE of low-FE pigs, while improved intestinal functions and decreased mitochondrial activity in the skeletal muscle are related to the high FE of high-ADG pigs.
4	Jiang et al., 2021	Gut microbiome and feed efficiency	Gut microbiome of low RFI pigs had a high abundance of the pathways related to amino acid metabolism and biosynthesis, but a low abundance of the pathways associated with monosaccharide metabolism and lipopolysaccharide biosynthesis. Propionic acid in feces and the serum metabolites related to amino acid metabolism were negatively correlated with the RFI.
5	McCormack et al., 2017	Intestinal microbiota and feed efficiency	In low RFI pigs had improved metabolic capabilities, especially within the ileal microbiota. Higher ileal isobutyric acid concentrations were also found in low RFI pigs. The differences observed within the intestinal microbiota of low RFI pigs compared with that of their high RFI counterparts, suggest a possible link between the intestinal microbiota and FE in pigs.
6	McCormack et al., 2019	Intestinal microbiota and residual feed intake	Despite controlling genetics, diet specification, dietary phases, and management practices in each rearing environment, the rearing environment, encompassing maternal influence, herd health status, as well as other factors, appears to impact intestinal microbiota more than FE.
7	Metzler-Zebeli et al., 2018	Gene expression and feed efficiency	Results do not allow the determination of whether mucosal bacterial changes contributed to variation in FE or were rather a consequence of FE-related changes in the pig's physiology or feeding behavior.

[1]FCR, feed conversion ratio; SCFAs, short chain fatty acids; FE, feed efficiency; ADG, average daily gain; RFI, residual feed intake.

Table 3. Summary of the main subject and conclusions of the papers about microbiota versus high and low growth performance in pigs (continued)

Code	Study	Main study subject	Conclusion[1]
8	Oh et al., 2020	Gut microbiota and body weight	The structure of intestinal microbiota may affect growth traits in pigs through host-microbe interactions. Further in-depth studies will provide insights into how best to reshape host-microbe interactions in pigs and other animals as well.
9	Quan et al., 2018	Microbiome and feed conversion ratio	OTUs in the cecum and colon of the high FCR pigs might have a greater ability to utilize dietary polysaccharides and dietary protein compared to low FCR pigs, and the SCFAs and indolic compounds produced by microbial fermentation might improve porcine feed efficiency and promote intestinal health.
10	Quan et al., 2019	Fecal microbiota and feed efficiency	There was a different microbial community structure in the fecal microbiota of pigs with different feed efficiency. *Streptococcus gallolyticus* subsp. gallolyticus could be an important candidate microbe for improving FE. The fecal microbiota in high-FE pigs have a greater capacity to degrade dietary cellulose, polysaccharide, and protein and may have a greater abundance of microbes to promote intestinal health.
11	Quan et al., 2020	Metagenomic characterization and feed efficiency	Cecum microbiota in high FE pigs have slightly higher richness and evenness than low FE pigs. The species in the cecum of the high FE pigs have a greater ability to utilize dietary polysaccharides and proteins. Bacteria from the genus *Prevotella* might impair the establishment of a more effective nutrition harvesting microbiota because the interaction between them and other beneficial microbes.
12	Reyer et al., 2020	Host-Microbiota Interactions and feed efficiency	Due to an increased abundance of non-starch polysaccharide fermenting taxa, pigs with a low RFI might be more efficient in using feed components that resisted digestion in anterior intestinal segments. The involvement of general immunity pathways in low RFI pigs probably benefits FE through energy-saving mechanisms.

[1]FCR, feed conversion ratio; SCFAs, short chain fatty acids; FE, feed efficiency; ADG, average daily gain; RFI, residual feed intake.

Table 3. Summary of the main ideas and conclusions of the papers about microbiota versus high and low growth performance in pigs (continued)

Code	Study	Main study subject	Conclusion[1]
13	Si et al., 2020	Fecal microbiota and feed efficiency	*Clostridiales* and *Bacteroidales* were found to be potential early life predictive biomarkers for high FE. Predictive functional analysis also indicated that fecal microbes of the high FE pigs may have a high level of utilize dietary protein. The composition of fecal bacterial community was related to some host factors, especially litter size and parity.
14	Tan et al., 2017	Cecal microbiome and feed efficiency	There were differences in the cecal microbiota of individuals with different FE. Micro-organisms that differed in abundance were mainly related to carbohydrate metabolism and may affect the growth of the host. Functional analysis revealed that the differentially expressed genes affect the host's energy absorption mainly through the pathway of pyruvate-related metabolism.
15	Tan et al., 2018	Microbiota composition and feed conversion ratios	Potential biomarkers (genera) were found in different locations of the complete intestinal tract in the high and low FCR groups, which could be of potential use to distinguish individuals for growth efficiency. Metabolic pathways in different locations were different between the high and low groups because of the presence of different microbes.
16	Verschuren et al., 2018	Fecal microbial composition and feed efficiency	There is a diet and sex-dependent relationship between FE and the fecal microbial com-position at slaughter weight in grower-finisher pigs. FE might be improved by changing the nutrition of pigs partly through resulting changes in microbiota composition.
17	Vigors et al., 2020	Colonic microbiome and feed efficiency	Farm of birth has a substantial influence on microbial diversity and bacterial abundance in the pig colon, suggesting that the farm of birth is having a considerable impact on the long-term composition of the gut microbiome.
18	Wang et al., 2021	Variations in microbiota and feed efficiency	The various fecal and colonic microbiota of finishing pigs were correlated with different FE. The proportion of differentially abundant genes affects host metabolism. The pathways mediating the metabolism of cofactors and vitamins were significantly different between groups.
19	Yang et al., 2017	Fecal microbiota and feed efficiency	Gut microbiota might improve porcine FE through promoting intestinal health by the SCFAs produced by fermenting dietary polysaccharides and improving the utilization of dietary protein.

[1]FCR, feed conversion ratio; SCFAs, short chain fatty acids; FE, feed efficiency; ADG, average daily gain; RFI, residual feed intake.

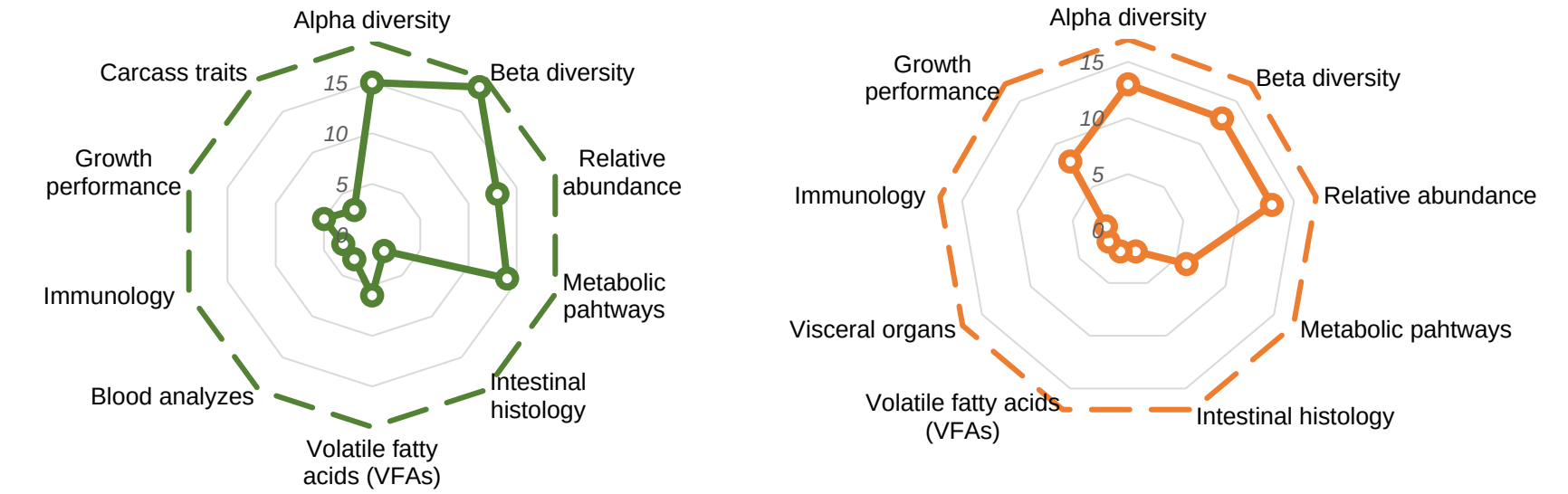

Figure 4. Categories evaluated in microbiota studies with high and low-performance in pig **(A)** and poultry **(B)** with dashed lines indicating the total number of publications included in each database.

Table 4. Summary of the studies to assess the microbiota with high and low performance on poultry production in terms of country, genetic, and sex.

Code	Studies	Country	Genetic	Sex
1	Du et al., 2020	China	Xiayan	Mixed
2	Huang et al., 2021	China	Yellow	Male
3	Lee et al., 2017	Republic of Korea	Ross 308	Mixed
4	Liu et al., 2021	USA	Cobb	Male
5	Lv et al., 2021	China	Yellow	Male
6	Metzler-Zebeli et al., 2019a	Austria	Cobb	Mixed
7	Metzler-Zebeli et al., 2019b	Austria	Cobb	Mixed
8	Shah et al., 2019	United Kingdom	Marshall	-
9	Siegerstetter et al., 2017a	Austria	Cobb	Mixed
10	Siegerstetter et al., 2018b	Austria	Cobb	Mixed
11	Siegerstetter et al., 2018c	Austria	Cobb	Mixed
12	Singh et al., 2012	India	Marshall	Male
13	Singh et al., 2014	India	Marshall	Male
14	Stanley et al., 2012	Australia	Cobb	Male
15	Stanley et al., 2013	Australia	Cobb	Male
16	Stanley et al., 2016	Australia	Cobb	Male
17	Yan et al., 2017	China	-	Female

Table 5. Summary of the methodologies applied to assess the microbiota in poultry with high and low growth performance

Code	Studies	Selection criteria[1]	Collection local	Intestinal tissue	Analysis method[2]	Region[3]
1	Du et al., 2020	RFI	-	Cecum	-	-
2	Huang et al., 2021	FCR	Digesta	Cecum	16S rRNA	V4
3	Lee et al., 2017	BW	Digesta	Cecum	16S rRNA	V4-V5
4	Liu et al., 2021	RFI	Luminal content	Ileum; Cecum; Cloaca	16S rRNA	V3-V4
5	Lv et al., 2021	FE	-	Duodenum; Ileum	16S rRNA	V3-V4
6	Metzler-Zebeli et al., 2019a	RFI	Digesta and mucosa	Ileum; Cecum	16S rRNA	V3-V5
7	Metzler-Zebeli et al., 2019b	RFI	Digesta and mucosa	Jejunum; Ileum; Cecum	16S rRNA	V3-V5
8	Shah et al., 2019	FCR	Luminal content	Small intestine[4]; ceca	16S rRNA	V3-V4
9	Siegerstetter et al., 2017a	RFI	Digesta and feces	Ileum; Cecum	16S rRNA	V3-V5
10	Siegerstetter et al., 2018b	RFI	Feces	-	16S rRNA	V3-V5
11	Siegerstetter et al., 2018c	RFI	Feces	-	16S rRNA	V3-V5
12	Singh et al., 2012	FCR	Feces	-	16S rRNA	V1-V5
13	Singh et al., 2014	FCR	Feces	-	Shotgun	-
14	Stanley et al., 2012	FCR	Digesta and mucosa	Jejunum; Cecum	16S rRNA	V1-V3
15	Stanley et al., 2013	AME	Digesta	Cecum	16S rRNA	V1-V3
16	Stanley et al., 2016	FCR	Digesta	Cecum	16S rRNA	V1-V3
17	Yan et al., 2017	RFI	Digesta and feces	Duodenum; Cecum	16S rRNA	V4

[1]Criterium selected to identify the different phenotypes in high and low performance; AME: Apparent metabolizable energy; RFI: residual feed intake; FCR: feed conversion ratio; BW: body weight; FE: feed efficiency.

[2]Analysis method for bacterial taxonomic classification; 16S rRNA: 16S rRNA gene sequencing; Shotgun: Shotgun metagenomic sequencing

[3]Hypervariable region of the bacterial 16S rRNA gene

[4]Collection in all segments of the small intestine

Table 6. Summary of the main subject and conclusions of the papers about microbiota versus high and low growth performance in poultry.

Code	Study	Main study subject	Conclusion
1	Du et al., 2020	Cecal microbial composition and feed efficiency	Identified a total of 22 potential biomarkers associated with FE, beneficial bacteria including *Lactobacillus* and *Limosilactobacillus oris*, and harmful bacteria such as *Campylobacter avium*, and *Helicobacter pullorum* in female and male chickens, respectively.
2	Huang et al., 2021	Cecal microbial composition and feed efficiency	Cecal microbiota has a possible connection with FE in yellow broilers. Bacteroides, may potentially be adopted as biomarkers for FE or used to modify dietary strategies for improving commercial poultry performance.
3	Lee et al., 2021	Cecal microbial composition by sex and body weight	Variation of cecal bacterial communities and their functions by sex and body weight may be associated with the differences in the growth potentials of broiler chickens.
4	Liu et al., 2021	Intestinal microbiota and residual feed intake	*Lachnospiraceae* family members are positively correlated with feed efficiency, other closely related bacteria have an opposite impact, highlighting a need to differentiate the bacteria to the species, subspecies, and even stain levels.
5	Lv et al., 2021	Microbial composition and feed efficiency	Microbial community structures in the duodenum and ileum of yellow broilers in the HFE and LFE groups was similar. Ileal microbiota is more correlated with FE than the duodenal microbiota. Isolation and culture experiments are needed to definitively demonstrate which bacteria can improve FE.
6	Metzler-Zebeli et al., 2019a	Intestinal microbiota and residual feed intake	The cecal abundance of *Anaerotruncus* was mainly associated with high RFI. Low-RFI chickens developed energy-saving mechanisms (i.e., shallower crypts and fewer goblet cells in ceca and a trend toward a lower-weight liver) and a stronger jejunal barrier function.

[1]RFI, residual feed intake; FMT, fecal microbiota transplant; FCR, feed conversion ratio; FE, feed efficiency, HFE, high feed efficiency; LFE, low feed efficiency.

Table 6. Summary of the main subject and conclusions of the papers about microbiota versus high and low growth performance in poultry (continuated)

Code	Study	Main study subject	Conclusion[1]
7	Metzler-Zebeli et al., 2019b	Fecal microbiota transplant and feed efficiency	The intestine only played a moderate role for the RFI-associated variation of the present low and high RFI phenotypes and may be related to energy-saving mechanisms, improved nutrient absorption and moderation of the mucosal immune response. The effects of the FMT were mostly independent from those of the RFI-associated variation in intestinal physiology and function, supporting the importance of host-specific factors for the observed RFI-associated variation.
8	Shah et al., 2019	Microbiome and feed conversion ratio	Gene transcription in low and high FCR sibs was significantly associated with the abundance of specific microbial taxa. Highly intertwined interactions between host transcriptomes and enteric microbiota are likely to modulate complex traits like FCR and may be amenable to selective modification with relevance to improving intestinal homeostasis and health.
9	Siegerstetter et al., 2017	Intestinal microbiota and residual feed intake	RFI-associated bacterial profiles could be identified across different geo-graphical locations. Results indicated that consortia of low abundance taxa in the ileum, ceca and feces may play a role for FE in chickens, whereby only bacterial FE-associations found in ileal and cecal digesta may serve as useful targets for dietary strategies.
10	Siegerstetter et al., 2018b	Fecal microbiota transplant and feed efficiency	Host and environment related factors may more strongly affect chicken fecal microbiota and FE than the fecal microbiota transplant.
11	Siegerstetter et al., 2018c	Fecal microbiota and Residual feed intake	Restrictive feeding-associated changes in the fecal microbiota were not similar in low and high RFI chickens, which may have been related to the higher nutrient retention and thus lower fecal nutrient availability in restrictively fed high RFI chickens.
12	Singh et al., 2012	Fecal microbiota and feed conversion ratio	In feed conversation ratio comparison of fecal bacteria, about 36 genera were differentially abundant between high and low FCR birds.

[1]RFI, residual feed intake; FMT, fecal microbiota transplant; FCR, feed conversion ratio; FE, feed efficiency, HFE, high feed efficiency; LFE, low feed efficiency.

Table 6. Summary of the main subject and conclusions of the papers about microbiota versus high and low growth performance in poultry (continuated)

Code	Study	Main study subject	Conclusion[1]
13	Singh et al., 2014	Fecal microbiome and feed conversion ratio	Poultry fecal metagenomes revealed the sequences related to 33 genera in both low and high FCR with significantly different proportion. Genes associated with sulphur assimilation, flagellum and flagellar motility were over represented in low FCR birds.
14	Stanley et al., 2012	Microbiota and feed conversation efficiency	Fecal metagenomic analysis of low and high FCR birds provided important insights into understanding of the taxonomic and functional potential of the poultry fecal microbiome. Significant differences in the two metagenomes indicated association of microbiome with phenotype. Genes associated with sulphur assimilation, flagellum and flagellar motility were over represented in low FCR birds.
15	Stanley et al., 2013	Microbiota efficiency of energy extraction	Among the phylotypes that were more prevalent in birds with high energy efficiency, most were closely associated with isolates of bacterial groups that are commonly recognized as producing enzymes that degrade cellulose and/or resistant starch. Phylotypes that were negatively correlated with performance were all unknown and uncultured, a significant number belonging to an unknown class of *Firmicutes*.
16	Stanley et al., 2016	Microbiota and bacterial identification	Even under controlled conditions different cohorts of birds developed distinctly different microbiotas. Within the different trial groups the abundance of certain bacterial groups correlated with productivity outcomes.
17	Yan et al., 2017	Gut metagenomic and feed efficiency	Functions relating to glycometabolism, and amino acid metabolism were enriched in the cecal microbiota of the better feed efficiency group. These results indicated the prominent role of cecal microbiota in the feed efficiency of chickens and suggested plausible uses of *Lactobacillus* to improve the feed efficiency of host.

[1]RFI, residual feed intake; FMT, fecal microbiota transplant; FCR, feed conversion ratio; FE, feed efficiency, HFE, high feed efficiency; LFE, low feed efficiency.

CAPÍTULO III

Effects dietary lysozyme levels on growth performance, body composition, blood profile and microbiota interaction in growing pigs.

Este capítulo é apresentado de acordo com as normas de publicação da **Plos One.**

Effects of dietary lysozyme levels on growth performance, body composition, nutrient balance, serum biochemical profile, and gut microbiota in growing pigs

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Abstract

Lysozyme is a natural anti-bacterial protein that is found in the saliva, tears, and milk of all mammals including humans. Its anti-bacterial properties result from the ability to cleave bacterial cell walls, causing bacterial death. This study was designed to evaluate a new fungal lysozyme product effect on growth performance, body composition, nutrient balance, blood profile, and microbial composition in growing pigs. Additionally, this study aimed to establish the optimal level of inclusion of lysozyme in the diet to maximize growth performance. Seventy-two barrows (40.6 ± 2.6 kg body weight [BW], Yorkshire × Landrace) were used for this experiment. Pigs were distributed in a completely randomized design with 12 replicates within six dietary treatments (0, 16, 32, 48, 64, and 80 mg of lysozyme/kg diet). Pigs were housed in the same pen, and individual transponders allowed feeders to identify the pigs, record their feed intake, and provide feed according to the individual assigned treatment. ADFI decreased linearly ($P \leq 0.01$), while ADG, G: F, PD, N, P and amino acids utilization efficiency increased linearly ($P \leq 0.01$) with the inclusion of lysozyme. Urea serum increased linearly ($P \leq 0.01$), whereas creatinine tended ($P \leq 0.09$) to decrease linearly and quadratically with increases in lysozyme supplementation levels. The minimal dietary lysozyme concentration maximizing ADG and G:F was 40 and 60 mg/kg, respectively. In jejunum digesta, Shannon and inverse Simpson indices showed significant effects ($P \leq 0.05$), whereas treatments did not impact Chao1 and ACE indexes, and there was no difference in differential abundance. Based on these results, if the aim is to maximize G:F, the ideal lysozyme inclusion level is 60 mg/kg. However, if, for diverse reasons the aim is to minimize enzyme inclusion while improving ADG, N utilization efficiency,

species evenness in jejunum digesta lysozyme supplementation from 40 up to 50 mg/kg of feed is recommended.

Key words: animal variability; enzyme; animal variability; muramidase

Introduction

Lysozyme (also called muramidase) is an enzyme naturally found in many mucosal secretions (tears, saliva, milk, and mucus) which has anti-inflammatory, immunological, and antibacterial properties (Sahoo et al., 2012). This enzyme has been studied as a potential alternative to antibiotics for livestock animals, and the effectiveness of lysozyme from different sources (from chicken eggs or obtained by biotechnological processes in milk or rice products) has also been evaluated in swine production (Brundige et al., 2010; Oliver and Wells, 2015; Long et al., 2016).

The use of some sources of lysozyme (from chicken eggs or obtained by biotechnological processes in milk or rice products) may be limited for industrial production due to volume requirements, economical limitations, or standardization restrictions. Fungal sources of lysozyme have been well-known for a long time (Osserman et al., 1974) but its standardized production is recent due to the advances in biotechnology. Fungal lysozyme is a promising candidate to enhance gut health (Larsen et al., 2021) and is a suitable alternative to growth-promoting antibiotic use in non-ruminant feed due to its capacity of improving the gastrointestinal microbiota (Oliver and Wells, 2015). However, still little is known about this viable source of lysozyme for non-ruminant animals.

However, studying feed additive supplementation can be limited by inherent individual animal variation. This variability results from differences among animals regarding intrinsic (e.g., genetic) and extrinsic (e.g., environmental) factors. Each animal responds uniquely to these factors, resulting in increased variability between animals (Wellock et al., 2004).

Due to the possible positive effect of lysozyme modulating the intestinal microbiota, and because of its anti-inflammatory properties, which may help protect the intestinal barrier and attenuate the immune response, we hypothesize that dietary lysozyme supplementation improve growth performance in pigs. Therefore, this study was designed to evaluate a new fungal lysozyme product effect on growth performance, body composition, nutrient balance, blood profile, and microbial composition in growing pigs. Additionally, this study aimed to establish the optimal level of inclusion of lysozyme in the diet to maximize growth performance in growing pigs and to assess whether there is a higher-respondent group for supplementation in heterogeneous populations.

Material and methods

Animals, housing, and management

Seventy-two barrows (40.6 ± 2.59 kg BW) of the same high-performance genotype (Yorkshire x Landrace) were used for the experiment. The animals were all in good health when they were shipped in single batch to the Agriculture and Agri-Food Canada research center in Sherbrooke, Quebec, Canada. Pigs were allocated in a 76 m^2 pen with concrete slat floors in the same mechanically ventilated room and had an ear

electronic chip granting them access to automatic feeding stations. Between arrival and the start of the trial, the pigs were fed commercial growing diets. Water was provided with low-pressure nipple drinkers, and feed was provided individually *ad libitum* throughout the adaptation period (14 d) due to health conditions that the animals arrived at the research center - without lysozyme supplementation - and experimental period (28 d) with 5 feeding stations (Automatic and Intelligent Precision Feeder; University of Lleida, Lleida, Spain). The temperature of the room was decreased gradually from 22°C when the piglets arrived at 18°C at the end of the experimental period to ensure thermoneutral conditions. The photoperiod consisted of 12 h of artificial light and 12 h of darkness. Health status was assessed daily, including observations of feed intake and monitoring for the presence of diarrhea and other signs of health disorders.

Pigs were distributed in a completely randomized design to the 6 treatments with increasing lysozyme levels (16, 32, 48, 64, and 80 mg/kg of diet). The experimental unit was the individual pig, and each treatment included 12 replicates. Pigs were housed in the same pen, and individual transponder codes allowed the feeders to identify individual pigs, record feed intake data, and the feeds to be provided to each pig according to the assigned treatments. In each single-space feeder, precision Archimedes screw conveyors delivered and simultaneously blended volumetric amounts of up to two feeds stored in independent containers located at the top of the feeder (Pomar et al., 2011). The feeder identified each pig when the feed demand was made, and the feeder read the specific treatment formula for that pig, mixed the feed in accordance with the assigned treatment, and dropped the feed into the feeder tray. A time lag between services was set in accordance with the pig’s BW and feed intake to

avoid cross-contamination. All the feeders were designed to provide meals to all the animals, regardless of the treatment. Therefore, all the animals could be housed in the same pen (Andretta et al., 2014; Andretta et al., 2016) and each animal could be considered an experimental unit. To avoid cross-contamination among the treatments, the rooms were cleaned twice a day and the feeders were calibrated weekly assuring the right treatment provision.

Experimental diets and treatments

The experimental pelleted feed was formulated on the basis of net energy and to meet or exceed standardized ileal digestible (SID) lysine (NRC, 2012). The other amino acid requirements were established in ratio to lysine, according to the ideal amino acid:lysine to maximize protein deposition (PD) (Gloaguen et al., 2013; Remus et al., 2019). Diet was free of antibiotics or zinc and copper at pharmacological levels (Table 1). Basal feed was divided into feed A (control) and B (lysozyme concentrated). Feed B consisted of the control feed supplemented with 300 mg/kg of lysozyme. Treatments were created by dilution, where automatic feeders mixed the right proportion of feed A and B for each treatment. The test was planned as a conventional dose-response study and consisted of a control group receiving a lysozyme-free diet (0 mg/ kg of diet), and 5 other groups receiving diets containing increasing lysozyme doses (16, 32, 48, 64, and 80 mg/kg of diet). This blend of feed A and B remained constant during the entire trial, and it was provided equally to all the animals within the same treatment.

The lysozyme product used in the study had the concentration of 128 g/L of lysozyme, and was produced by Concordia University, 1455 Boul. de Maisonneuve Ouest, Montréal, Canada.

Body composition and nutrient balance

Total body fat, lean, bone mineral content, and bone mineral density were measured by dual X-ray (DXA) on the first experimental day and 21 days later with a densitometry device (GE Lunar Prodigy Advance, Madison, WI, USA). Pigs were scanned in a prone position using the Total Body scanning mode (Lunar enCORE Software Version 8.10.027). Anesthesia was induced with sevoflurane (7%) and maintained with isoflurane (5%) during the scans. The DXA body lean, and fat mass values were converted to their protein and lipid chemical equivalents as proposed by Kipper et al. (2019). Individual protein deposition (PD, g/d) was estimated by the difference between the predicted protein masses on days 21 and 0. Body phosphorus (P) was obtained assuming a constant distribution of phosphorus in the whole body and bone ash (Nielson, 1973). Nitrogen (N) and P excretion values were obtained by subtracting the respective nutrient retention values from the nutrient intake values. Lysine and threonine efficiency of utilization above maintenance were calculated (van Milgen et al., 2008) assuming a fixed amino acid concentration in the PD. The choice of scanning the animals 7 days before the end of the trial (slaughter) was made to enable the selection of low and high responders in terms of PD. As well, due to a logistic question once scanning and sacrificing animals on the same day would not be feasible.

Growth Performance

Pigs were weighed on arrival, once during the pre-experimental phase for animal selection, and, on days 0, 14, 21, and 28. Animal growth performance was evaluated through average daily feed intake (ADFI, kg/d), average daily gain (ADG, kg/d), gain-to-feed ratio (G:F, kg/kg), amino acid efficiency of utilization for PD (%), PD (g/d), PD in daily gain (%), and lipid deposition (LipD, g/d). Due to experimental constraints, pigs were fed manually in the last 7 days of the trial, and ADFI was not recorded.

Feces sampling

Diarrhea scores were recorded, and feces samples were collected on days 0, 14, 21, and 28. Feces samples were collected by rectal stimulation during the weighing of all pigs. The feces samples were stored directly into a whirl pack bag, kept on ice, and immediately separated into aliquots for dry matter (DM) and microbiota analysis. Each fresh sample was given a fecal score according to the consistency and appearance using the fecal consistency score system, where 0 = normal feces, 1 = soft feces, 2 = mild diarrhea, and 3 = severe diarrhea (Pérez-Calvo et al., 2019). All fecal samples were kept at -20˚C during the sampling period (maximum 2 hours) and then stored at -80˚C and -20˚C for microbiome and DM analysis respectively, at the end of the day until future analyses.

Blood and tissue sampling

On day 28, 48 animals were selected in relation to their PD (low or high) for slaughter. Blood samples were collected into 20 mL tubes near the jugular vein (mixture of venal and arterial blood) and centrifuged at 1800×*g* for 12min at 4°C to recover serum samples. Liver tissue was weighed (without the gallbladder), and a vein-free standardized sample was collected from the right lobe and stored at -80°C. Segments (middle point of the small intestine, counting the total number of turns of tissue and dividing by half the total number of turns) and caecum (distal part of the tissue, the content of the digesta was pressed towards the upper part and an incision was made with scissors) were collected to determine microbiome of the digesta and mucosa scraping for gene expression analysis. The samples were sub-sectioned at 10 cm lengths of standardized tissue and the digesta of each tissue were collected separately. Measurements of the pH of the digesta samples were determined using an electronic pH meter. Sections of the jejunum and caecum tissue were rinsed with ice-cold phosphate buffered saline (pH 7.4), sliced longitudinally using a scalpel and scraped mucosa using RNAse-free glass slides into sterile tubes and snaped frozen in liquid nitrogen for later storage at -80°C until analysis. All samples were collected and frozen within 20 min of exsanguination.

Measurements in serum samples

Thirteen parameters were measured in serum samples, including urea, creatinine, cholesterol, triglycerides, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, globulin, albumin/globulin ratio, IgG and

protein C-reactive (CRP) were used to evaluate health and nutritional status of animals. Concentrations of IgG in blood were determined through ELISA kits (Pig IgG ELISA Quantification Set, ref. E100-104; Bethyl Laboratories, Inc., Place, Country). The biochemical and enzymatic analyses of blood serum were performed using an automatic analyzer (Beckman Coulter AU680 and AU5800 models, Brea, CA, USA).

DNA extraction and 16S rDNA sequencing

Total DNA was extracted from fecal, jejunum, and cecum samples. Microbial profiling was performed using high-throughput sequencing of the V3-V4 region of the 16S rRNA gene.

Statistical analysis

Growth performance analyses were performed using the R package software (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria). Growth performance and carcass data were analyzed as a complete randomized design using the lmerTest package (Kuznetsova et al., 2017), considering the individual pig as the experimental unit. The main fixed effect was the lysozyme dose, and initial body lipids was used as covariate in all growth performance and nutrient balance analyses. The assumption of normal distribution of the error, influential values, and outliers presence were checked using the performance package (Lüdecke et al., 2021). The uncertainty in the estimate of the means of the data was expressed as the maximum standard error (MSE), and a P-value less or equal than 0.05 was statistically significant, whereas a P-value greater than 0.05 and less than 0.10 was considered a tendency. Differences

between individual treatments were analyzed using polynomial contrasts from base R, and estimated marginal means (Seare et al., 1980) were obtained with the package emmeans of R. The optimal lysozyme dose was assessed with linear and curvilinear-plateau models using the nlstools (Baty et al., 2015) and rcompanion (Mangiafico, 2016) packages of R. The frequency of diarrhea was calculated creating a binary variable (0=scores 0 or 1 vs 1=scores 2 or 3) from the fecal scores and analyzed with a frequency table. For DM feces was used polynomial contrasts with lmerTest package in R program.

Analyses of the fecal, cecal, and jejunal microbiota were performed in R. Beta diversity (Aitchison distance) was calculated using package vegan, while alpha-diversity indices (Observed ASVs, Chao1 index, Abundance Based Coverage Estimator (ACE), and Inverse Simpson index) were calculated with package phyloseq. Differential abundance was calculated with Microbiome Multivariable Associations with Linear Models (MaAsLin2) package. The model was run using Centered Log-Ratio (CLR) normalization and LM method, while False Discovery Rate (FDR) was calculated with Benjamini–Hochberg method. Lysozyme dose and sample type (for digesta) or experimental day (for feces) were considered fixed effects and the individual animals were considered random effects. The reference levels for the contrasts were defined as control for the variable Lysozyme, Day 0 for the variable experimental day in feces samples, and cecum for the variable sample type in digesta samples. Adonis function of the vegan package was used to perform Non-parametric MANOVA (PERMANOVA) to test the effect of the Lysozyme dose and sample type (for digesta) or experimental day (for feces) and their interaction on the beta-diversity. The coefficients resulting from the

PERMANOVA were used to identify the taxa most responsible for the variation between groups. Packages ggplot2 and ggpubr were used for plots and figures.

Results

Growth performance

The growth performance responses were evaluated over three periods (1-14 days,1-21 days, and 1-28 days), and body composition was evaluated on days 1 and 21 of the experiment (Table 2).

Animals were randomly distributed in the treatments. For that reason, some differences among the initial condition could be found. No difference in initial BW was observed among treatments. However, the initial body protein and P mass tended ($P \leq 0.10$) to increase in a quadratic manner, and the initial body lipid mass tended ($P \leq 0.10$) to increase linearly across treatments. Thus, the initial body lipids mass was used as a covariate in all growth performance analyses, being significant in most of the models used.

The ADFI decreased linearly ($P \leq 0.01$) as the lysozyme level in the diet increased in both studied periods (1-14 or 1-21 days). Conversely, the ADG and G:F increased linearly ($P \leq 0.01$) with the dietary lysozyme levels during the same periods. Linear and quadratic effects of lysozyme levels were found ($P \leq 0.05$) for the BW at 14 and 21 days. However, no effect of lysozyme levels on BW was found on the 28th day of the trial with only a tendency of linear effect ($P = 0.07$) observed for ADG in this later period.

The protein deposition during the trial and body protein mass on the 21st day also increased linearly (P ≤ 0.05) as the dietary lysozyme level increased. Otherwise, lipid deposition and body lipid mass on the 21st day of the trial also increased quadratically (P ≤ 0.05) with increases in dietary lysozyme levels. No effects of lysozyme levels were found for the body phosphorus or calcium masses.

Nutrient balance

Nutrient balance (1-21d) was positively affected by dietary lysozyme levels (Table 3). The reduction in ADFI previously described resulted in a linear (P ≤ 0.01) decrease in the consumption of SID lysine, and crude protein as dietary lysozyme increased. As ADG and BW were positively affected by the enzyme, a linear (P ≤ 0.01) increase in the efficiency of lysine and threonine utilization was observed.

In the same way, the decrease (P ≤ 0.01) in N and P intake and the improvement in its retention (P ≤ 0.05) resulted in a linear increase in N and P efficiency (P ≤ 0.01) as the level of lysozyme in the diet increased. Consequently, N and P excretions were also linearly reduced (P ≤ 0.01) by the lysozyme.

Serum biochemical profile

Serum urea increased linearly (P ≤ 0.01), whereas creatinine tended (P ≤ 0.09) to decrease linearly and quadratically with increases in lysozyme supplementation levels (Table 4). Alanine aminotransferase also decreased linearly (P ≤ 0.10) with the lysozyme levels.

Fecal dry matter and frequency of diarrhea

Dry matter and frequency of diarrhea of growing pigs are presented in Tables 5 and 6, respectively. No difference in fecal dry matter and frequency of diarrhea were observed among treatments throughout the experimental period.

Estimation of optimal lysozyme dose

The optimal lysozyme dose was estimated using the linear and quadratic-plateau models considering the G:F and ADG responses (Fig. 1). For ADG, the breakpoint of the linear-plateau model was observed at the lysozyme level of 40.2 mg/kg of feed ($P < 0.01$; CI: 5.2 to 75.3 mg/kg) for a maximal ADG of 996 g (CI: 954 to 1038 g). The quadratic-plateau model estimated the ideal lysozyme level of 50.2 mg/kg of feed ($P < 0.01$; CI: -11 to 112 mg/kg) for a maximal ADG of 990 g (CI: 945 to 1035 g).

For G:F, the breakpoint of the linear-plateau model was observed at the lysozyme level of 62.4 mg/kg of feed ($P < 0.01$; Confidence interval (CI): 45.6 to 79.3) for a maximal G:F of 0.46 (CI: 0.44 to 0.49). The breakpoint of the quadratic-plateau model was estimated above maximal supplementation in this trial, and therefore not presented.

Exploring variation in the growth response to the treatment

Aiming to explain part of the variation as a response to the treatments, the protein deposition was regressed as a function of lysozyme intake considering or not

initial body fat as a covariate. Lysozyme levels alone, explained only 10% of the variation of protein deposition (R^2 = 0.10), whereas lysozyme level and initial lipid body content (% of BW) explained together 27% of the variation of PD (R^2 = 0.27).

For G:F, the percentage of variation explained by the treatment increased from 64 to 68% by adding initial body lipid percentage as a covariate in the model. Finally, no change in the percentage of variation explained by the treatment on ADG was observed by adding body lipid percentage as a covariate.

Microbial diversity in intestinal and fecal samples

There were no differences in alpha diversity between treatments in fecal samples. As for the digesta samples (jejunum and cecum), Shannon and inverse Simpson indices showed significant effects ($P \leq 0.05$) for the treatment effect, in which it was possible to observe a lower uniformity in the jejunum in relation to the cecum and decreased from the supplementation of 32 mg/kg of dietary lysozyme, indicating that the bacterial groups in the treatment groups had different proportions, whereas in the control treatment, the bacterial groups were more equally populated. For the Chao1 and ACE (richness index) no difference was observed (Fig 2).

Beta-diversity analysis in feces and digesta samples

Principal component analysis (PCA) based on Aitchison distance showed no significant impact of the treatment on the distribution of samples in all matrices analyzed, indicating that the microbial composition was very similar in each sample regardless of treatment (Fig. 3). However, the type of sample (feces, jejunum, and

cecum) had a significant effect for the Aitchison and Bray-Curtis distance tested. A clear separation among the fecal samples analyzed in different days (Aitchison distance) was found, in which the day zero of the experimental period differed from the other periods ($P < 0.001$; **Supplementary Figure S1**).

Differential abundance in feces and digesta samples

No differences were identified in the overall abundance at the phylum level for fecal and digesta samples. For fecal samples, at the family level, only *WCHB1.41_fa* tended to decrease ($P = 0.06$) in the group fed 64 mg/kg of lysozyme. At the genus level, *Lachnospiraceae_AC2044_group* decreased in the treatments fed 32 mg/kg ($P = 0.044$) and 64 mg/kg ($P = 0.011$), while *Sarcina* tended to decrease at 32 mg/kg ($P = 0.058$) and 80 mg/kg ($P = 0.079$) of dietary lysozyme. However, *UCG.009* decreased significantly in 64 mg/kg ($P = 0.028$) of lysozyme, while *Turicibacter* and *Clostridium_sensu_stricto_1* tended to decrease at 32 mg/kg ($P = 0.078$; $P = 0.099$, respectively), and *Alloprevotella* and *Escherichia shigella* tended to increase at 32 mg/kg and 48 mg/kg ($P = 0.099$) in comparison to the treatment control (**Table 7**). Within the family level of digesta samples, *Firmicutes_unclassified* had increased abundance at 80 mg/kg ($P = 0.032$) and a tendency to increase at 48 mg/kg ($P = 0.096$) of lysozyme. At the genus level, *Firmicutes_unclassified* increased at 80 mg/kg ($P = 0.045$), while *UCG.002* tended to increase at 32 mg/kg ($P = 0.064$), and *Jeotgalicoccus* decreased at 32 mg/kg ($P = 0.054$) of lysozyme in relation to treatment control (**Table 8**).

Discussion

In this study, dietary supplementation of lysozyme tended to enhance the growth performance of growing pigs by improving feed efficiency (G:F) during the 14 and 21d periods. The improved G:F ratio is due to the linear increase in ADG and linear decrease in ADFI. Although the literature is limited regarding the effect of fungal dietary lysozyme on growth performance of growing pigs, previous studies have demonstrated greater ADG, G:F, BW, and villus height to crypt depth ratio, which was attributed to improvements in gut morphology and microbiota (May et al., 2012; Long et al., 2016; Zou et al., 2019).

Moreover, the linear decrease in lysine, threonine, and crude protein intake allowed the animals to be more efficient for both amino acids, as dietary lysozyme levels increased. The improvement in the N and P balance as dietary lysozyme increased are likely due to the lower nutrient intake and excretion. As nutrient supply was constant, the effect is due to the direct impact of lysozyme in ADFI. Previous studies have reported contradictory effects of lysozyme in feed intake. No effect on feed intake was observed when lysozyme was supplemented to pigs at 50 or 100 mg/kg in the diet in addition to antibiotics (Zou et al., 2019), or supplemented at 100 mg/kg in the diet compared to antibiotics (Oliver et al., 2015), while other study found increased dietary intake with lysozyme (Deng et al., 2021). In this study, we found a decrease in feed intake as the level of lysozyme in the diet increased, which may be related to an improvement in nutrient absorption, particularly amino acids, for which an improved efficiency was found (Michael and Nathan, 2000).

A dose-response effect appeared between (G:F, 62.4 mg/kg of feed - linear-plateau model; ADG, 40.2 mg/kg of feed - linear plateau model and 50.2 mg/kg of feed - quadratic plateau) indicating the optimal level of supplementation of this new source of lysozyme to improve growth performance in growing pigs. Choosing the ADG variable, as it is a direct measure of animal growth performance would facilitate understanding the growth rate, its ability to reach market weight in a given period, and how to manage the improvement in feeding and health status with an optimal level (up 40 mg/kg) of lysozyme known. While the G:F variable strongly influences financial returns (Gaines et al., 2012), due to its close association with feed costs, it becomes a variable of great interest to the animal production industry.

Lysozyme supplementation also linearly improved PD and increased final body protein mass, while quadratically decreasing final body lipid mass. These results, together with the increase in serum urea and decrease in creatinine serum levels support the hypothesis that lysozyme can increase PD likely due to a better protein utilization efficiency, linked to changes in protein intake and metabolism. Previous studies in pigs have shown that the decrease in muscle catabolism correlates with a decrease in serum and plasma urea levels (Davis et al. 2004; Bush et al. 2002). Our data do not corroborate this finding, and due to changes in protein intake and PD, is likely that the increase in urea in the serum might be correlated to greater amino acid absorption, once blood samples were obtained two hours after the last meal. Moreover, creatinine tended to decrease quadratically with increasing lysozyme supplementation, differing from previous studies that measured the effect of lysozyme on creatinine and found no significant response (Zou et al., 2019; Schliffka et al., 2019 and Zhou et al.,

2019). Creatinine is produced when creatine phosphate is broken down in muscle (Wyss and Kaddurah-Daouk 2000), and serum creatinine levels are positively correlated with muscle mass (Hosten, 1990). Thus, the measured increase in serum urea, along with the increased efficiency of DP and N utilization, creatinine levels found in this study may also be related to dietary protein origin and tissue protein turnover. Positive changes in the microbiota, immune response and oxidative stress likely improve protein metabolism in pigs supplemented with lysozyme (Xiong et al., 2019). As a previous study (Zhou et al., 2019), alanine aminotransferase decreased linearly as the level of lysozyme in the diet increased. This suggests that pigs that received higher levels of lysozyme had less liver cell damage, and therefore indicating that lysozyme had a hepatic protective effect, since alanine transaminase is an indicator of hepatocellular injury (Ekser et al., 2012).

Lysozyme improved growth performance and nutrient efficiency. A series of studies have proven the beneficial effects of dietary supplementation of lysozyme on the growth performance of pigs at different stages of growth, including 10-day-old pigs (May et al., 2012) weaned pigs (Long et al., 2016), and growing pigs (Zou et al., 2019). Most studies attribute the improvement in growth performance to improvements in gut morphology, in which a higher VH:CD in the jejunum was observed for animals that received higher dietary lysozyme supplements (Zou et al., 2019; Long et al., 2016; Oliver and Wells, 2013). In this particular study, the increase in urea levels in blood serum observed that consumed higher levels of lysozyme may have contributed to the improvement in the growth performance of the animals. This suggests that pigs receiving higher levels of dietary lysozyme could be using dietary AA for protein

synthesis more efficiently than control animals, corroborating higher protein deposition and greater efficiency in nutrient use (e.g., amino acids and nitrogen) found in this study.

More important than understanding the effect of lysozyme on animal growth performance, body composition, and nutrient balance is understanding the factors behind the differences between animals. In this trial, we observed a variation within treatments for the variables analyzed in this study. This variation within the treatment is much more associated with the variation between animals, regarding the body composition and metabolic factors of the individuals than with the treatment effect. This is because the percentage of body lipids considered in the model as a covariate is significantly involved in the variation in body composition among pigs. One of the responsible factors that may partially explain this animal variability is insulin sensitivity since insulin is a positive regulator of fatty acid synthesis and body adiposity (Weickert et al., 2006). Salgado et al. (2022), clearly showed that insulin sensitivity is negatively correlated with total body lipids in pigs and that insulin sensitivity explained about 45% of the variations in total body lipids and proteins among pigs. Therefore, insulin sensitivity is an important factor in determining dietary energy and nutrient utilization with implications for body composition in growing pigs, being a factor in determining animal variability should be considered.

Consistent with previous studies (Zou et al., 2019; Xu et al., 2020) the lysozyme affected the evenness in digesta (Shannon and Simpson indexes) as lysozyme in the diet increased, while richness indexes were not impacted (Chao1 and ACE indexes), indicating there were uniformity changes between treatments. In our study, this lower

uniformity could be observed when the animals received the treatment 32 mg/kg of lysozyme in the diet, indicating that the bacterial groups in the treatment groups had different proportions, while the animals that did not receive lysozyme supplementation in the diet the bacterial groups were more equally populated. The main coordinate analysis also indicated that there were no differences in overall diversity between treatments, which indicated that the microbial composition of the animals was very similar in each sample, regardless of the treatment. This is related to our finding that no significant differential abundances were found. We observed that there is only microbial participation in some specific treatments (most of the effects due to the 32 and 80 mg/kg of lysozyme in feces and digesta, respectively), which does not alter the general composition of the microbiota.

According to Yegani and Korver (2008) and Vigors et al., (2020), several factors such as diet, environment, and genetics induce changes in the intestinal microbiota; in this study, enzyme use is one of the most important factors tested. However, alterations in the intestinal microbiota were not observed. Potential factors in our study were strictly controlled, as the experimental conditions were in perfect condition, the animals were in good health, i.e., without any stressors that could challenge the effect on the action of lysozyme. In this case, it is very difficult to find changes in these factors under controlled conditions, and most feed additives work in more challenging situations such as commercial farms. This enzyme influenced the decrease in species uniformity with the increase in lysozyme, which depending on the proportionality or uniformity between groups of bacteria could contribute to the results of growth performance with significant responses in growth performance as the dose of lysozyme in the diet increased and not

for the microbiota, the path may lie in the functionality of communities. In this sense, more studies that address the metabolic pathways are needed to better understand the dynamics of the microbiota for this specific source of fungal lysozyme.

Conclusions

Growth performance and nutrient balance in this trial were affected by the inclusion of lysozyme in the diet. Growing pigs decreased ADFI, while improving ADG, G:F and PD as dietary lysozyme increased. Lysozyme supplementation improved the efficiency of lysine and threonine utilization, as well as increased N and P utilization, due to changes in ADFI and nutrient excretion. While changes in diversity and relative abundance of specific commensals were seen, there were no clear alterations in the populations as a result of increasing levels of lysozyme. The maintenance of diversity with increased inclusion rates would support the health and growth of animals during early life. Based on these results, if the aim is to maximize G:F, the ideal lysozyme inclusion level is 60 mg/kg. However, if, for diverse reasons the aim is to minimize enzyme inclusion while improving ADG, N utilization efficiency, species diversity in jejunum digesta lysozyme supplementation from 40 up to 50 mg/kg of feed is recommended.

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Table 7. Ingredients and nutritional composition of the experimental feed to pigs (40 - 64 kg BW) during the growing phase.

Items	
Ingredients (as-fed basis), g/kg	
Corn, ground	556.46
Wheat	150.00
Soybean meal	250.00
Fat	7.00
Limestone	15.50
Sodium bicarbonate	5.50
Monocalcium phosphate	3.92
Salt	1.90
Vitamin-mineral premix[1]	2.00
L-Lysine HCL	3.90
DL-Methionine	1.50
L-Threonine	1.70
L-Tryptophan	0.32
Choline chloride	0.20
Phytase[3]	0.10
Estimated chemical composition, %	
Dry matter	87.05
Crude protein	18.01
SID[2] Lysine	1.10
SID Methionine	0.39
SID Threonine	0.72
SID Tryptophan	0.22
SID Histidine	0.42
SID Isoleucine	0.64
SID Leucine	1.32
SID Phenylalanine	0.32
SID Valine	0.72
SID Cysteine	0.29
Ca	0.74
Total P	0.42
Digestible P	0.30
Ca : P	2.33
Net energy (MJ/kg)	10.05

[1]Each kilogram of feed phase I provides at least the following nutrients: vitamin A, 9999 IU; vitamin D, 499 IU; vitamin E, 44 IU; vitamin K, 0.78 mg; vitamin B12, 0.02 mg; niacin, 14 mg; pantothenic acid, 11.57 mg; pyridoxine, 0.83 mg;

riboflavin, 3.17 mg; thiamine, 1.26 mg; copper, 121 mg; iodine, 0.29 mg; iron, 361 mg; manganese, 85 mg; selenium, 0.3 mg; zinc, 164 mg. [2]SID = Standardized Ileal Digestible; [3]Quantum Blue 500

Table 8. Body composition and growth performance of growing barrows (40 - 64 kg BW) fed different inclusion levels of lysozyme enzyme for each period.

	Lysozyme levels						P-values		
Expected, mg/kg	0	16	32	48	64	80	SEM[1]	Lin[2]	Quad[3]
Number of observations	12	12	12	12	12	12			
Day 0									
BW[4], kg	39.93	40.29	41.01	41.38	40.79	40.57	0.47	0.45	0.15
Body protein mass, kg	8.13	8.23	8.37	8.43	8.29	8.25	0.09	0.59	0.08
Body lipid mass, kg	2.38	2.27	2.36	2.40	2.54	2.51	0.10	0.09	0.53
Body P mass, g	205.62	208.27	209.75	216.30	207.35	207.39	3.29	0.64	0.07
Body Ca mass, g	267.57	271.69	271.8	284.57	268.16	269.16	5.83	0.83	0.13
Days 1 to 14									
Final BW, kg	54.12	55.31	56.18	57.13	55.34	56.49	0.61	0.01	0.04
ADFI[5], kg/day	2.22	2.07	2.13	1.79	1.74	1.75	0.04	<0.001	0.33
ADG[6], kg/day	1.01	1.04	1.08	1.13	1.05	1.15	0.03	<0.01	0.71
Gain : feed ratio	0.46	0.5	0.51	0.63	0.60	0.65	0.02	<0.001	0.49
Days 1 to 21									
Final BW, kg	61.7	63.61	64.08	64.93	63.46	64.09	0.69	0.03	0.02
ADFI, kg/day	2.25	2.08	2.17	1.88	1.75	1.84	0.04	<0.001	0.29
ADG, kg/day	1.03	1.09	1.09	1.12	1.10	1.13	0.02	0.01	0.34
Gain : feed ratio	0.46	0.53	0.50	0.60	0.62	0.62	0.01	<0.001	0.15
Protein deposition, g/day	182.51	192.01	187.66	193.38	189.26	200.77	4.93	0.03	0.80
Lipid deposition, g/day	200.08	214.7	239	219.14	221.73	215.48	10.65	0.38	0.05
Protein in weight gain, %	17.59	17.54	17.17	17.72	17.50	17.76	0.19	0.42	0.19
Final body protein mass, kg	11.97	12.30	12.33	12.60	12.23	12.44	0.15	0.05	0.09
Final body lipid mass, kg	6.60	6.91	7.43	7.01	7.07	6.93	0.22	0.38	0.05
Final body P mass[7], g	323.34	331.33	332.00	340.27	328.79	332.63	5.38	0.29	0.15
Final body Ca mass[8], g	435.99	445.79	446.31	458.7	442.03	445.94	9.75	0.53	0.26
Days 1 to 28									
BW, kg	71.27	71.48	73.05	73.74	71.58	72.81	1.00	0.30	0.28
ADG, kg/day	1.09	1.08	1.13	1.14	1.11	1.16	0.03	0.07	0.95

[1]SEM = standard error of the mean.
[2] Lin = probability of linear effect.
[3] Quad = probability of quadratic effect.
[4] BW = Body weight.
[5] ADFI = average daily feed intake.
[6] ADG = average daily gain.
[7]P = Phosphorus.
[8]Ca = Calcium.

Table 9. Nutrient efficiency of growing barrows (40 - 64 kg BW) fed different inclusion levels of lysozyme enzyme for 21 days.

	Lysozyme levels						P-values		
Expected, mg/kg	0	16	32	48	64	80	SEM[1]	Lin[2]	Quad[3]
Number of observations	12	12	12	12	12	12			
Nutrient balance									
Lysine intake (SID[6]), g/day	23.60	21.68	22.69	19.77	18.57	19.50	0.48	<0.001	0.22
Lysine efficiency, %	53.62	62.59	57.61	70.29	70.57	71.50	2.37	<0.001	0.38
Threonine (SID), g/day	15.44	14.18	14.84	12.93	12.15	12.76	0.31	<0.001	0.22
Threonine efficiency, %	44.60	52.06	47.92	58.46	58.69	59.47	1.97	<0.001	0.38
Total protein intake, g/day	385.64	354.3	370.77	323.08	303.48	318.71	7.85	<0.001	0.22
N[4] intake, g/day	64.64	59.39	62.15	54.15	50.87	53.42	1.32	<0.001	0.22
N retention, g/day	29.29	31.11	30.18	30.93	29.95	31.87	0.85	0.15	0.97
N excretion, g/day	35.49	28.28	31.97	22.36	20.92	21.56	1.56	<0.001	0.21
N efficiency, %	45.55	52.8	48.65	59.11	59.27	60.12	1.99	<0.001	0.41
P[5] intake, g/day	9.45	8.68	9.08	7.91	7.43	7.81	0.19	<0.001	0.22
P retention, g/day	5.60	5.79	5.79	5.90	5.85	5.99	0.14	0.05	0.71
P excretion, g/day	3.85	2.89	3.29	2.01	1.59	1.82	0.20	<0.001	0.15
P efficiency, %	59.56	66.97	63.92	75.06	78.95	77.32	1.94	<0.001	0.33

[1]SEM = Standard error of the mean.
[2] Lin = Linear.
[3] Quad = Quadratic.
[4] N = Nitrogen.
[5] P = Phosphurus.
[6] SID = Standardized ileal digestibility.

Table 10. Serum biochemical profile in growing barrows (40 - 64 kg BW) fed different inclusion levels of lysozyme enzyme during 28 days.

Parameter	Lysozyme levels						P-value		
	0	16	32	48	64	80	SEM[1]	Lin[2]	Quad[3]
Number of observations	12	12	12	12	12	12			
Urea, µmol/L	6.99	6.63	7.10	7.05	7.62	7.80	0.35	0.01	0.33
Creatinine, µmol/L	91.58	88.75	82.67	84.00	83.33	85.29	2.90	0.06	0.09
Cholesterol, mmol/L	2.51	2.51	2.46	2.63	2.59	2.61	0.08	0.18	0.91
Triglycerides, mmol/L	0.54	0.51	0.49	0.54	0.59	0.51	0.05	0.77	0.86
Glucose, mmol/L	5.36	5.46	5.50	5.48	5.43	5.64	0.16	0.35	0.92
Aspartate aminotransferase, U/L	90.5	69.83	77.75	80.92	77.17	70.98	13.09	0.51	0.83
Alanine aminotransferase, U/L	57.17	53.17	54.92	52.33	51.50	51.91	2.43	0.10	0.60
Total protein, g/L	64.2	63.87	64	64.76	63.42	63.89	0.93	0.78	0.82
Albumin, g/L	38.83	39.14	38.13	39.51	38.69	38.66	0.66	0.88	0.88
Globulin, g/L	25.37	24.72	25.65	25.25	24.73	25.23	0.83	0.87	0.99
Albumin/globulin ratio	1.54	1.60	1.50	1.59	1.58	1.56	0.06	0.77	0.97
IgG[4], µg/mL	6.21	5.92	7.03	6.10	6.91	6.22	0.44	0.56	0.41
CRP[5], ug/mL	200.19	205.11	178.74	181.70	168.31	195.41	23.24	0.49	0.44

[1]SEM = standard error of the mean.
[2] Lin = linear.
[3] Quad = quadratic.
[4] IgG = immunoglobulin.
[5] CRP = Porcine C-Reactive Protein.

Table 11. Fecal dry matter (%) of growing barrows (40 - 64 kg BW) fed different inclusion levels of lysozyme enzyme for 28 days.

Item	Lysozyme levels, mg/kg						*P-values*		
	0	16	32	48	64	80	SEM[1]	Lin[2]	Quad[3]
DM[4] (%)									
Day 0	26.42	27.85	27.23	25.39	27.21	26.78	0.96	0.79	0.95
Day 14	26.52	27.80	25.61	27.74	27.67	27.73	0.84	0.27	0.75
Day 21	27.90	29.69	28.30	28.91	29.26	29.05	0.69	0.38	0.63
Day 28	29.32	29.38	26.17	30.26	28.74	27.14	1.21	0.39	0.89

[1]SEM = standard error of the mean.
[2] Lin = linear.
[3] Quad = quadratic.
[4] DM = Dry Matter

Table 12. Frequency of diarrhea (%) of growing barrows (40 - 64 kg BW) fed different inclusion levels of lysozyme enzyme for 28 days.

Item	Lysozyme levels, mg/kg						Total[2]	*P-values*
	0	16	32	48	64	80		
Binary variable[1]	0	0	0	0	0	0	0	
Frequency of diarrhea, %								
Day 0	83.33	91.67	81.82	83.33	83.33	91.67	61	0.83
Day 14	91.67	91.67	91.67	91.67	91.67	83.33	65	0.56
Day 21	100	100	100	100	100	100	72	-
Day 28[3]	100	100	87.50	100	100	87.50	46	0.40
Total period	93.18	95.45	90.70	93.18	93.18	90.91	244	0.60

[1]Binary variable: 0 = absence of diarrhea (scores 0).
[2]Total number of observations per binary variable throughout the experimental period.
[3]Values representative of the number of animals selected for protein deposition.

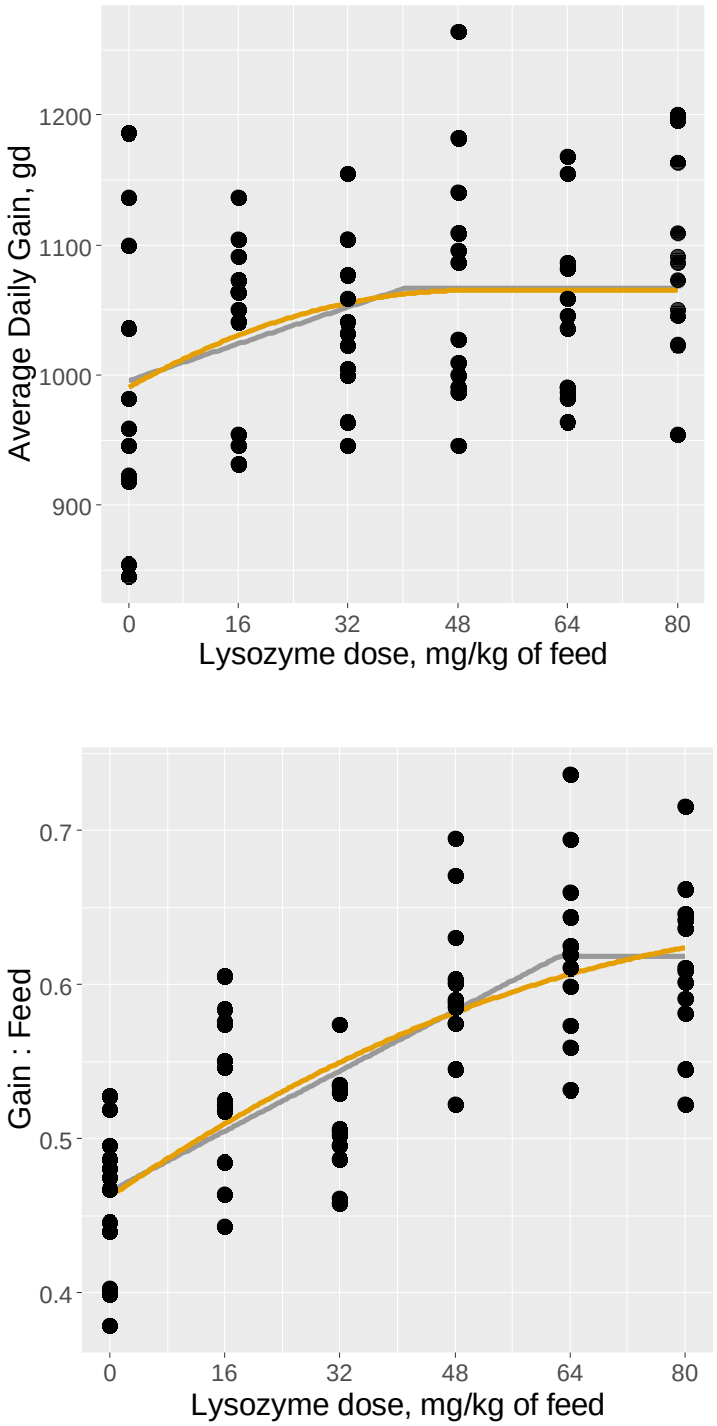

Fig. 5 Average daily gain (ADG) (kg/day) according to the linear-plateau and quadratic plateau models for growing pigs at 21 days

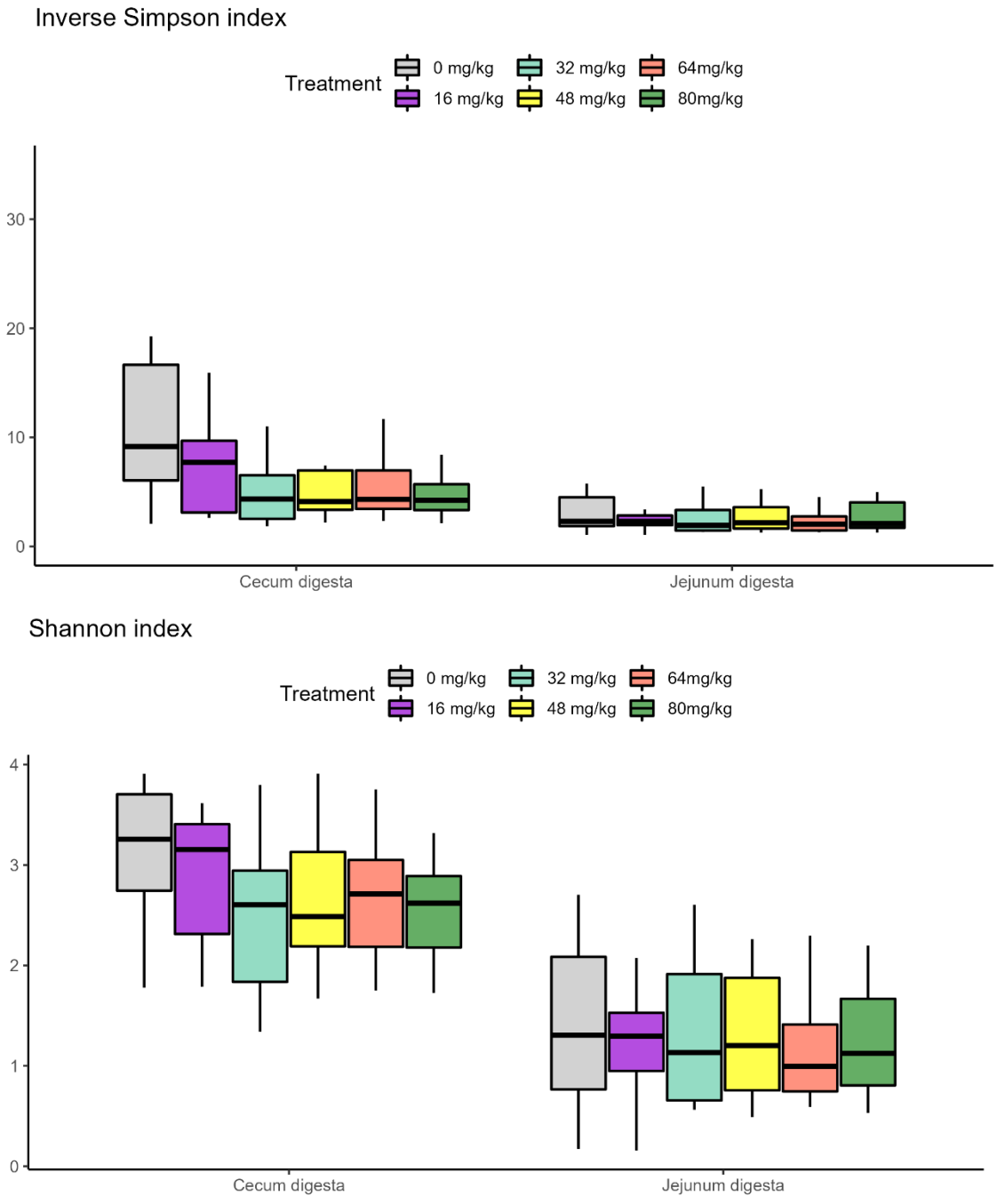

Fig. 6 Diversity index of the microbiota in growing pigs at different levels of lysozyme.

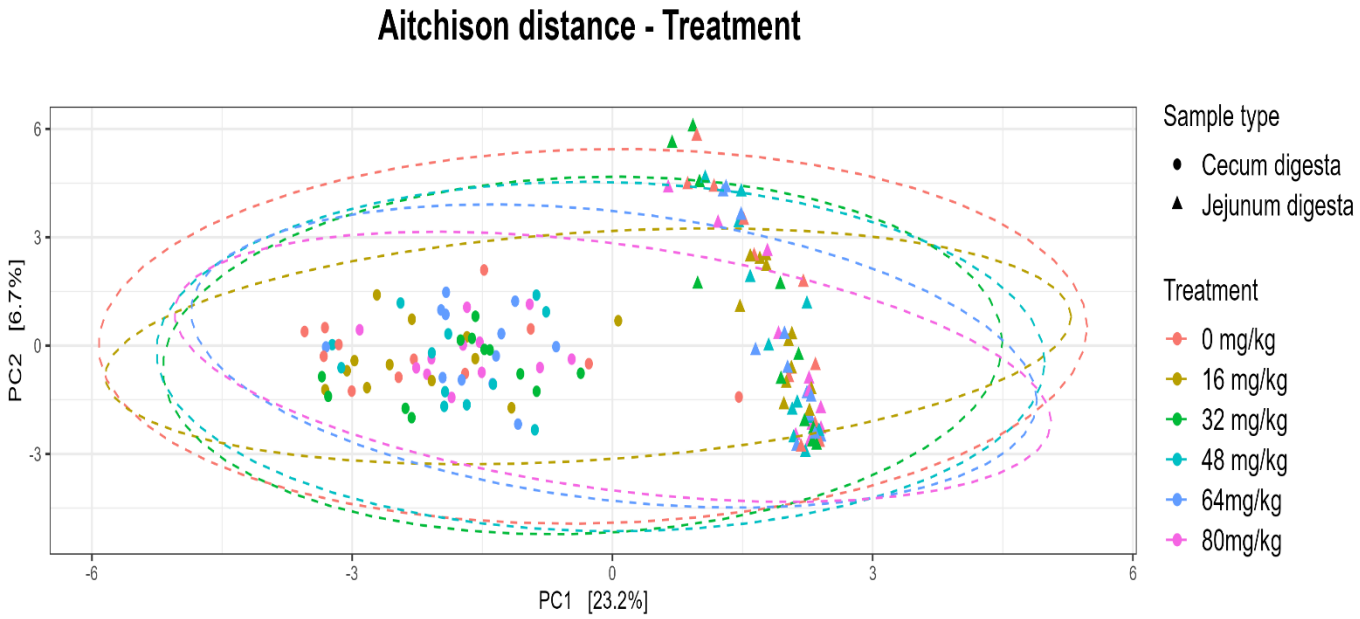

Fig. 7 Beta-Diversity of the microbiota in growing pigs at different levels of lysozyme

Table 13. Differential abundance for treatment effect compared to the control treatment in feces samples for growing pigs.

Variables	Trt[1]	Coef[2]	SE[3]	P value
Phylum:				
No effect				
Family:				
WCHB1.41_fa	64	-0.609	0.232	0.060
Genus:				
Lachnospiraceae_AC2044_group	64	-1.129	0.344	0.011
UCG.009	64	-0.672	0.228	0.028
Lachnospiraceae_AC2044_group	32	-0.952	0.343	0.044
Sarcina	32	-1.230	0.465	0.058
Turicibacter	32	-0.747	0.297	0.078
Sarcina	80	-1.167	0.465	0.079
Alloprevotella	32	0.605	0.252	0.099
Clostridium_sensu_stricto_1	32	-0.684	0.284	0.099
Escherichia.Shigella	48	0.425	0.176	0.099

Data correspond to 287 samples; [1]Treatments; [2]Coefficient value model (size effect); [3]Standard error.

Table 14. Differential abundance for treatment effect compared to the control treatment in digesta samples for growing pigs.

Variables	Trt[1]	Coef[2]	SE[3]	P value
Phylum:				
No effects				
Family:				
Firmicutes_unclassified	80	0.827	0.282	0.032
Firmicutes_unclassified	48	0.717	0.282	0.096
Genus:				
Firmicutes_unclassified	80	0.812	0.291	0.045
Jeotgalicoccus	16	-0.470	0.172	0.054
UCG.002	32	0.424	0.159	0.064

Data correspond to 143 samples; [1]Treatments; [2]Coefficient value model (size effect); [3]Standard error.

4 CONSIDERAÇÕES FINAIS

Este trabalho mostrou a partir da revisão sistemática, que a microbiota intestinal dos animais, juntamente com os fatores abordados, pode explicar em parte as diferenças no desempenho zootécnico de suínos e aves com alta e baixa eficiência alimentar. Um dos principais pontos é que há variação significativa entre os estudos em relação aos critérios de seleção para determinação de animais com diferentes fenótipos (alto e baixo desempenho) e nas metodologias para determinação da microbiota. Além disso, esses estudos nos direcionam para algumas alternativas viáveis para melhorias na eficiência alimentar animal, como por exemplo a modificação da composição da dieta e melhorias na eficiência do uso de vias metabólicas.

Por outro lado, a lisozima melhorou o desempenho animal, composição corporal e o balanço de nutrientes. Suínos em crescimento diminuíram ADFI, enquanto melhoraram ADG, G:F e PD à medida que a lisozima dietética aumentou. A suplementação com lisozima melhorou a eficiência de utilização de lisina e treonina, bem como aumentou a utilização de N e P. Não houve alterações significativas nas populações da microbiota intestinal com o aumento dos níveis de lisozima. Além disso, para máxima G:F o nível ideal de inclusão de lisozima é de 60 mg/kg, enquanto para a eficiência de utilização de ADG e N, recomenda-se de 40 a 50 mg/kg de ração.

A realização desta pesquisa fez parte de um grande projeto multidisciplinar e possibilitou trabalho com áreas distintas da produção animal, contribuindo para um aprendizado mais dinâmico e interdisciplinar. Este trabalho agrega conhecimento sobre o uso de aditivo alimentar com potencial antibacteriano e anti-inflamatório para saúde intestinal em suínos e fatores que afetam a variabilidade animal, principalmente a microbiota animal, fatores que estão potencialmente envolvidos na variação da resposta observada à ingestão nutricional e suplementação com aditivo alimentar, uma área que carece de informações científicas. Este trabalho deixa além de um aprendizado científico, questionamentos e ideias que podem continuar a serem explorados.

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VITA

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